Intravitreal Injection of TGFβ Induces Cataract in Rats

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PURPOSE. In a previous study, it was determined that TGFβ induces cataractous changes in the rat lens in vitro. The purpose of the present study was to determine whether the introduction of biologically active TGFβ into the vitreous stimulates cataractous changes in the rat lens in situ.

METHODS. TGFβ was injected into the vitreous of the left eye of anesthetized adult male Wistar rats. The right eye received sterile vehicle as a control. Three to four months after injection, animals were killed, and lenses were enucleated and examined for cataractous changes.

RESULTS. All lenses from control eyes remained transparent and maintained normal cellular architecture throughout. In contrast, lenses from TGFβ-injected eyes displayed cloudiness in the cortex. In some lenses, distinct opacities were also apparent at the equator and extending some distance toward the anterior and posterior poles. Histologically, the opacities corresponded to subcapsular plaques containing aberrant cells and accumulations of extracellular matrix. In addition, cortical fibers in the anterior and posterior of all lenses displayed variable degrees of swelling, and many retained their nuclei. In some regions, the fiber cells appeared to have degenerated to form large homogenous areas. The cellular architecture of the equator of these lenses was also disrupted and, in the most severe case, no bow zone was apparent with nucleated cells extending to the posterior pole.

CONCLUSION. The introduction of active TGFβ into the vitreous induced lenses to undergo cataractous changes. In addition to the TGFβ-induced changes in the epithelium that were reported previously, cataractous changes observed in this study also involved the lens fiber cells and resembled changes observed in human posterior subcapsular and cortical cataracts. (Invest Ophthalmol Vis Sci. 1999;40:3231–3236)

Previously, we reported that TGFβ stimulates lens epithelial cells to undergo aberrant morphologic and molecular changes that mimic those documented in some forms of human cataract. In lens epithelial explants TGFβ induces capsule wrinkling, the formation of spindle-shaped cells that contain α-smooth muscle actin and cell death.1,2 Furthermore, distinct anterior opacities develop in whole lenses cultured with TGFβ.3,4 Histologically, the opacities correspond with subcapsular plaques, which contain spindle-shaped cells and accumulations of extracellular matrix, including type I collagen.5 Morphologic and molecular features similar to those induced by TGFβ in vitro have been described in human anterior and posterior subcapsular cataract and in aftercataract (posterior capsular opacification).5–13 These findings have led to the hypothesis that TGFβ may be involved in the etiology of these forms of cataract.

Analysis of ocular media of patients who are to undergo cataract surgery suggests that TGFβ levels are elevated compared with ocular media collected from eye bank eyes.14,15 However, it is not clear whether the elevated level of TGFβ in these patients was the inducing factor for the cataract or a consequence of the cataract. The purpose of this study was to investigate whether elevating active TGFβ levels in the ocular media in vivo induces cataractous changes in the lens similar to those previously reported in studies in vitro. A whole animal model was used, in which TGFβ was injected into the vitreous of adult male rats. Lenses from these animals have been shown to be both highly responsive and sensitive to the cataractogenic influence of TGFβ.4 Here, we describe the cellular and molecular changes that are induced in the lens by intravitreal injection of TGFβ.

MATERIALS AND METHODS

Human recombinant TGFβ2 from two different sources was used in this study. TGFβ from Genzyme (Cambridge, MA) was supplied in 30% acetonitrile-0.1% trifluoroacetic acid and used undiluted. TGFβ (R&D Systems; Minneapolis, MN) was supplied lyophilized in a 0.1% very low endotoxin fraction V bovine serum albumin (BSA) carrier and was resuspended according to the manufacturer’s instructions in sterile 2-mM HCl-phosphate buffered saline (PBS)-0.5% insulin-free BSA (Sigma, St. Louis, MO).

Intraocular Injection Protocol

All experimental procedures used in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and
Vision Research. Thirteen adult Wistar rats (9-months old) were used in this study. Because adult male rats had been shown to be highly susceptible to the cataractogenic influence of TGFβ, these animals were exclusively used in this study. Each rat was anesthetized using 5% halothane in 70% NO2-30% O2 then maintained in 1.5% halothane in the same gas mixture for the duration of all surgical procedures. The rat was positioned on its right side on an operating table under a dissecting microscope with its left eye uppermost. Using a fine needle (Insulin Syringe, Becton Dickinson, Franklin Lakes, NJ; external diameter, 360 μm), a small puncture was made in the region of the limbus. A very fine needle (Hamilton, Reno, NV; external diameter, 200 μm) attached to a 10-μl syringe was immediately lowered through the puncture hole into the vitreous using a micromanipulator, and 3 μl (approximately 60 ng) TGFβ, was slowly injected into the vitreous. The needle was left in position for 30 to 60 seconds and then slowly withdrawn to minimize fluid loss from the eye. Injections were performed under a dissecting microscope to ensure correct positioning of the needle and to monitor loss of fluid from the eye. It should be noted that the maximum effective dose of TGFβ to which lens cells were exposed as a result of this procedure is unknown. Injection would generate a positive pressure, which would probably lead to displacement of some TGFβ out of the vitreous compartment. In addition, the outcome would be influenced by inhibitors of TGFβ known to be present in the vitreous.16

After injection, each animal was placed in a warmed (26°C), humidified recovery box and allowed to regain consciousness. Each rat was then anesthetized for a second time, and the same protocol was used to inject the right eye with 3 μl control vehicle, either acetonitrile-trifluoroacetic acid or HCl-phosphate-buffered saline (PBS)-BSA (depending on the source of TGFβ). Rats were monitored regularly (at least twice weekly) for infection associated with the injection site. Most rats were killed at 15 weeks, one was killed at 6 weeks, and another was killed at 12 weeks. No time-dependent differences were noted.

Collection of Lenses and Tissue Processing

After the appropriate period after injection, rats were euthanized by CO2 asphyxiation, and eyes were removed and placed in culture dishes containing Medium 199 (Trace Biosciences, Sydney, Australia) with BSA and antibiotics. Both eyes from four rats were fixed whole for routine histology to determine the influence of TGFβ on all ocular tissues. The lenses were removed from remaining eyes, as described for whole lens cultures,3 examined for the presence of opacities, and photographed. Representative lenses and whole eyes were fixed in Carnoy’s (1:3 acetic acid:ethanol) and processed for routine histology and immunolocalization. Briefly, entire lenses and whole eyes were serially sectioned and collected onto subbed glass slides. Every fifth slide was stained with hematoxylin-eosin, and sections were examined for evidence of plaque formation and/or fiber deterioration. Sections corresponding to those that displayed histologic changes were used for immunolocalization of β-crystallin and the extracellular matrix proteins laminin and type I collagen. Nuclei were counterstained (Hoechst 33258; Boehringer-Mannheim, La Jolla, CA).

RESULTS

All lenses from eyes injected with TGFβ displayed some loss of lens clarity and changes in cellular architecture, although the response varied from lens to lens. Note that none of the lenses in this study, control or TGFβ-treated, displayed evidence of rupture of fibers through the lens capsule, as would happen if the needle tip had penetrated the capsule during the injection procedure. Furthermore, when sections of control and TGFβ-treated eyes were examined histologically, no differences in extralenticular tissues were detected.

Influence of TGFβ on Lens Transparency

For every rat examined in this study, the lens from the right eye (vehicle-treated control) remained transparent (Fig. 1A). Of the nine lenses dissected from eyes that were injected with TGFβ, four showed distinct subcapsular opacities (for example, Fig. 1B). In these lenses, the opacities tended to be localized in a region around the lens equator but also extending some distance toward the anterior (Fig. 1B) and/or posterior (Fig. 1C) poles of the lens. A generalized more diffuse clouding, which appeared to involve the cortical fiber cells, was a feature of all lenses from eyes injected with TGFβ. This was most readily

![Figure 1](https://i.imgur.com/123456789.png)

**FIGURE 1.** Influence of TGFβ in vivo on lens transparency. Micrographs of lenses from eyes injected with vehicle (A) or TGFβ (B, C). Rats were killed 15 weeks after intravitreal injection. Lenses were carefully dissected from surrounding ocular tissues in culture medium and photographed. The lenses in (A) and (C) were photographed through the posterior pole, and the lens in (B) was photographed through the anterior pole. The lens from the control eye had remained transparent (A); however, lenses from TGFβ-injected eyes had distinct opacities (*arrows*), predominantly at the equator, which extended anteriorly (B) and posteriorly (C). Lenses from TGFβ-injected eyes also showed diffuse clouding in the cortical fibers (B). Scale bar, 400 μm.
detected in the region surrounding the reflection of the light source (for example, see Fig. 1B).

**TGFβ-Induced Changes in the Equatorial Region**

Histologic examination of serial sections revealed that all control lenses maintained a normal lens equator and bow zone (Fig. 2A). In contrast, of the 10 lenses from eyes injected with TGFβ examined histologically, in 6 the characteristic appearance of the lens equator and bow zone was disrupted (for example, Fig. 2B). Nucleated fiber cells were commonly observed in the lens cortex and extending toward the posterior pole. These lenses also tended to lose their uniform curvature, as depicted by the undulating capsule in Figure 2B.

**TGFβ-Induced Changes in the Anterior Region**

Control lenses exhibited normal cellular architecture. They maintained a monolayer of epithelial cells overlying the highly aligned and tightly packed fiber cells (Fig. 3A). Of the nine lenses dissected from eyes injected with TGFβ, eight showed some degree of opacification in the anterior region. Discrete opacities corresponding with subcapsular plaques were present in six of the nine lenses (for example, Figs. 1B, 3B). In other lenses, opacities were associated with a thickening of the lens capsule and general disruption of the epithelium (Fig. 4A). Regardless of the form of the subcapsular plaque the surrounding fiber cells appeared swollen and degenerative and no longer maintained alignment or packing (Fig. 3B). Deeper into the cortex, fiber cells tended to degenerate and form large homogeneous regions. In addition, swollen fiber cells, immediately adjacent to the lens epithelium and deeper in the cortex, often retained their nuclei. These cells have a similar appearance to that of the swollen bladder cells described in posterior subcapsular cataract.

TGFβ-induced subcapsular plaques contained abnormal cells. In some plaques the cells appeared rounded and contained large, dense nuclei. In others, where matrix deposits were a prominent feature, cells appeared flattened and slightly elongated except for a layer of epithelial-like cells beneath the matrix deposits. In addition, smaller matrix deposits were commonly observed between the epithelial cells across the anterior surface of the lenses between the plaques. Immunolocalization revealed that the subcapsular plaques contained deposits of laminin and type I collagen (Figs. 3D, 3F). Strong reactivity for laminin was also detected in the lens capsule. In addition, a fine band of reactivity for laminin was often detected at the interface between the plaques and fiber cells (Fig. 3D). Type I collagen was detected predominantly in the subcapsular plaques (Fig. 3F), although very weak reactivity was sometimes associated with epithelial cells between the plaques. In lenses from control eyes, laminin was detected only in the lens capsule, and no specific reactivity for type I collagen was detected (Figs. 3C, 3E).

Another form of anterior opacification was also observed. A large vacuole was observed from the lens capsule at the anterior pole deep into the cortical fibers (Fig. 4A). The overlying capsule displayed evidence of remodeling; nucleated cells appeared to be embedded in the extracellular matrix of the capsule, which was irregular in its thickness, forming a small residual plaque (Fig. 4A). The fibers surrounding this vacuolated region and associated plaque were very swollen and highly disrupted, with complete loss of regular alignment (Fig. 4B). It is interesting to note that this is the only rat in which opacification of the lens in the TGFβ-injected eye was apparent in vivo.

**TGFβ-Induced Changes in the Posterior Region**

Nucleated cells were observed at the posterior pole of some of the lenses from eyes injected with TGFβ. Nuclei were present in fiber cells located directly adjacent to the posterior capsule and extending deeper into the cortex (Fig. 5B). The nucleated fiber cells often appeared enlarged and swollen, reminiscent of bladder cells. In some areas these cells appeared to have degenerated forming homogeneous regions in the cortex (Fig. 5D). No nucleated, swollen, or degenerating cells were detected in the posterior of any of the control lenses (Figs. 5A, 5C).

**Discussion**

Previous investigations, both in vitro and using transgenic mice, have suggested a role for TGFβ in the formation of
subcapsular cataracts. In the present study, a whole-
animal model was used to investigate the effect of introducing
active TGFβ into the vitreous. A variety of morphologic and
molecular changes resulted that were similar to changes asso-
ciated with several common forms of cataract in humans.

Intravitreal injection of TGFβ induced disruption of the
normally highly aligned arrangement of the fiber cells. Fibers
appeared swollen and retained their nuclei, and in some re-
gions there was evidence of degeneration of fibers into
rounded fragments and large homogeneous areas. Swollen,
degenerating fibers were apparent from the anterior through
the equator and into the posterior of the lens. TGFβ also
induced severe disruption of the ordered arrangement of nu-
clei at the lens equator and in the bow region of these lenses.
Furthermore, many fiber cells atypically retained their nuclei
beyond the bow region. In all these respects, changes in lenses

![Figure 3](image-url) **Figure 3.** Influence of TGFβ on cells anterior to the lens equator. Serial sagittal sections of lenses from eyes injected with vehicle (A, C, and E) or TGFβ (B, D, and F) were viewed by phase-contrast microscopy (A, B) or used for immunofluorescence localization of laminin (C, D) or type I collagen (E, F). Control lenses retained normal packing and alignment of the fiber cells (A). In lenses exposed to TGFβ, subcapsular plaques developed (B). The fibers surrounding the plaques appeared swollen, and some retained their nuclei (arrowheads), while in other areas fibers appear to degenerate and form large homogeneous regions (asterisk). The control lens displayed a normal distribution of laminin in the lens capsule (C) and no specific reactivity for type I collagen (E). The lens exposed to TGFβ showed strong reactivity for laminin in the lens capsule and in matrix-like deposits in the subcapsular plaques (D). A fine band of laminin reactivity was also detected at the plaque-fiber interface (arrowheads). Strong reactivity for type I collagen was also associated with the matrix-like deposits in the subcapsular plaques (F). Scale bar, 24 μm.

![Figure 4](image-url) **Figure 4.** Influence of TGFβ on cells at the anterior pole of the lens. Sagittal sections of lenses from an eye injected with TGFβ were stained with hematoxylin-eosin (A) or used for immunolocalization of β-crystallin and nuclear counterstaining (B). A large vacuole was observed at the anterior pole extending from beneath the epithelium into the cortex (A). A residual plaque was located above the vacuole with cells embedded within the lens capsule, which was irregular and uneven in its thickness (A, arrow). The fiber cells surrounding the vacuole appeared disrupted and swollen. Immunolocalization of β-crystallin revealed the extent of the swelling and disruption in the fiber cells (B). Stained nuclei were unevenly distributed in the region overlying the vacuole (B, arrows). Scale bar, (A) 50 μm; (B) 60 μm.
from eyes injected with TGFβ resemble those associated with human cortical cataract.20,21

Lenses from eyes injected with TGFβ also displayed changes characteristic of posterior subcapsular cataract. In lenses exposed to TGFβ, nucleated cells were observed extending from the disrupted equator to the posterior pole of the lens as described in posterior subcapsular cataract.17,22 Furthermore, swelling and deterioration of the fiber cells extending from the posterior capsule to deep within the cortex of the lens, another feature of posterior subcapsular cataract, was common.

Epidemiologic studies indicate that posterior subcapsular cataract and cortical cataract commonly occur together in mixed cataracts.23–25 This has resulted in some confusion in establishing exactly which morphologic features arise in each type. Nevertheless, swelling and deterioration of the fiber cells extend- ing from the disrupted equator to the posterior pole of the lens as described in posterior subcapsular cataract.17,22 For example, both these forms of cataract develop numerous distinct opacities across the anterior surface that correspond with subcapsular plaques.3,4 Some subtle changes in the posterior and cortical fibers have been observed previously in lenses cultured with TGFβ but only in lenses from ovariectomized rats, which are particularly susceptible to the cataractogenic effects of TGFβ.4

Several factors may contribute to the observed differences in the effects of TGFβ on the lens in vivo and in vitro. In studies in vitro, the entire lens is immersed in medium containing TGFβ. In contrast, in the present study in vivo, TGFβ was introduced directly into the vitreous chamber; therefore, the initial and presumably greatest TGFβ insult was inflicted on the posterior region of the lens. Another factor may be the duration of the experiment. In studies in vitro, the time that lenses may be cultured without deterioration is limited (up to 7 days for lenses from adult rats), whereas lenses were generally not examined until 15 weeks after exposure to TGFβ in vivo. TGFβ-induced changes, either direct or secondary, may take longer to manifest in cortical fibers than in epithelial cells. Cells would also have more time to migrate posteriorly along the capsule in the study in vivo.

Differentiating fiber cells in the cortical region have the potential to respond directly to TGFβ. TGFβ receptors have been detected by immunolocalization and in situ hybridization in these cells as well as in the equatorial region and the lens epithelium.26 However, the observed swelling of cortical fibers may be a secondary response, the result of osmotic imbalance due to TGFβ-induced disruption of the epithelium. This phenomenon does not appear to be the result of nonspecific damage associated with the injection procedure, because no such changes were observed in control lenses. Further investigation is required to determine the precise mechanisms involved in the cortical fiber response.

TGFβ is known to be present in the ocular environment. The ocular media contain TGFβ and both mRNA and protein have been detected within the lens.16,18 Normally, TGFβ bioavailability to the lens appears to be tightly regulated. Mechanisms that contribute to suppression of TGFβ activity include its production in a latent form and binding to regulatory molecules.27,28,16 Recent work in this laboratory has indicated that the ocular media contain inhibitors of TGFβ. Vitreous in particu-lar has been shown to strongly inhibit TGFβ activity, dem-

**Figure 5.** Influence of TGFβ on cells at the posterior pole of the lens. Sagittal sections of lenses from eyes injected with vehicle (A, C) or TGFβ (B, D) were stained with hematoxylin-eosin (A, B) or left unstained and viewed by phase-contrast microscopy (C, D). No nucleated cells were present at the posterior pole of the control lens (A) and the fiber cells were aligned and tightly packed (C). The lens exposed to TGFβ had numerous nuclei present at the posterior pole (B, arrowheads). The fiber cells in this region appeared swollen and rounded and were no longer aligned (D). Nuclei appeared as rounded or elongated bright areas (D, arrowheads). Scale bar, (A, B) 40 μm, (C, D) 25 μm.
onstrated by its effectiveness in blocking the morphologic changes induced by TGFβ in lens epithelial explants in vitro. At least part of the inhibitory activity of the ocular media may be attributable to the presence of α-2-macroglobulin.

Although the ocular media contain TGFβ inhibitory molecules, it is unclear that their effects can be overcome. For example, in the present study rats that received a single intravitreal injection of TGFβ subsequently developed cataracts. Similarly, in humans it has been reported that patients who received intracocular TGFβ to promote healing of macular holes later exhibited an unusually high incidence of cataract. Furthermore, transgenic mice that overexpress TGFβ specifically in the lens developed subcapsular cataracts. Elucidating the mechanisms and molecules that are involved in regulating TGFβ activity in the ocular media, and in the eye in general, represents an important area for future study that may lead to new strategies for preventing or slowing TGFβ-induced cataract.

The present study adds further support to the hypothesis that TGFβ is involved in the etiology of subcapsular cataract, in both the anterior and posterior hemispheres of the lens. In addition, the results indicate that TGFβ may be involved in the etiology of at least some cortical cataracts. Having now established this in vivo model for TGFβ-induced cataract, it will be possible to use it in future studies to assess the effects of cataract prevention strategies in the whole animal.

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References