Susceptibility to TGFβ2-Induced Cataract Increases with Aging in the Rat

Angela M. Hales, Coral G. Chamberlain, and John W. McAvoy

PURPOSE. Cataract is the most common cause of blindness in the world today, and yet there is no generally accepted treatment other than surgical intervention. Studies in rodent models designed to increase understanding of the molecular basis of cataract have shown that transforming growth factor (TGF)-β induces morphologic and molecular changes similar to those associated with some forms of human cataract. Because aging is the most widely recognized risk factor for cataract, it is important that any animal model be examined in this context. This was a study of the effects of aging on susceptibility to TGFβ-induced cataract.

METHODS. Lenses from weanling, adult, and senile rats were cultured in defined serum-free medium with a range of concentrations of TGFβ2. The lenses were cultured for up to 7 days, photographed daily, fixed, and prepared for histology and immunolocalization. Opacification was quantified by image analysis.

RESULTS. Lenses from weanling, adult, and senile rats all underwent similar cataractous changes when exposed to TGFβ. This included opacification, the formation of anterior subcapsular plaques, and accumulation of type I collagen and α-smooth muscle actin. Lenses from adult and senile animals, however, were generally more adversely affected by TGFβ than lenses from weanlings. This study also showed that a low dose of TGFβ administered over a prolonged period had an effect similar to that of a higher dose administered over a shorter period.

CONCLUSIONS. An elevation of TGFβ activity, either acute or chronic, and/or an age-related increase in lens cell susceptibility to TGFβ may be triggering factors in the etiology of certain forms of cataract.

Cataract is the leading cause of blindness worldwide.1 For example, cataract accounted for nearly half the 38 million cases of blindness recorded in a recent World Health Organization survey.2 Despite the extent of the problem and considerable research effort over the years, there is no generally accepted pharmacologic agent that can be applied to prevent or slow the onset or progression of this disease. Development of such a therapeutic agent depends on increased understanding of the molecular basis of cataractogenesis.

In this laboratory we have been investigating the effects of various growth factors on lens biology and disease. We have shown that in vitro all three mammalian isoforms of transforming growth factor (TGF)-β induce responses in lens cells that mimic events in cataractogenesis. Lens epithelial cells in explants3,4 and cultured whole lenses5 are induced to undergo molecular and morphologic changes that are typically associated with subcapsular cataracts and with aftercataract, the subcapsular opacification that often arises from lens cells remaining after cataract surgery.6 TGFβ induces distinct anterior subcapsular opacities in cultured lenses from weanling rats.5 These opacities correspond histologically with subcapsular plaques, which contain aberrant cells and are virtually indistinguishable from early-stage anterior subcapsular cataract in humans. Lenses and lens explants cultured with TGFβ express molecular markers for subcapsular cataract, type I collagen, and α-smooth muscle actin.5,6 In addition, when TGFβ2 was injected into the vitreous, cortical and posterior subcapsular opacities developed in the lenses, and they showed histologic changes characteristic of these forms of cataract in humans.7 Taken together, these findings point to a role for TGFβ in the etiology of major forms of cataract.

A variety of risk factors predispose toward cataract, but the most widely recognized of these is aging.1,8 For example, prevalence of cataract approximately doubles with each decade after 30 years of age. The reason for this age-related increase is not clear. However, because such a strong link exists, it is imperative that any animal model for human cataract be examined in the context of aging. In the present study, lenses from rats of various ages were compared in terms of their susceptibility to the cataractogenic effects of TGFβ. The influence of different exposure regimens was also assessed. Lenses were cultured with a range of concentrations of TGFβ and the rate and extent of opacification were quantified. The onset of key morphologic and molecular markers for human cataract was also monitored.

From the Department of Anatomy and Histology and the Institute for Biomedical Research, The University of Sydney, New South Wales, Australia.

Supported by Grant R01 EY03177 from the National Eye Institute, National Institutes of Health, and the National Health and Medical Research Council of Australia. AH was the recipient of a Faculty of Medicine Postgraduate Scholarship and a U2000 Postdoctoral Research Fellowship, both from The University of Sydney.

Submitted for publication June 28, 1999; revised December 29, 1999 and January 31, 2000; accepted February 15, 2000.

Commercial relationships policy: N.

Corresponding author: John W. McAvoy, Save Sight Institute, PO Box 4337, Sydney, NSW 2001, Australia.
MATERIALS AND METHODS

All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Wistar rats were used, and lenses were obtained from 21- to 22-day old rats of either sex (weanlings), 6- to 10-month-old ex-breeding-colony males (adult), or 2.5-year-old males (senile). Whole lens were dissected from any adherent vitreous material and maintained in 4 ml serum-free M199 medium (Trace Biosciences, Sydney, Australia) containing 0.1% bovine serum albumin (Sigma, St. Louis, MO), 0.1 µg/ml l-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, and 2.5 µg/ml amphotericin B (Fungizone; all from Trace Biosciences), as described previously.5 Whole lenses were cultured with the anterior surface, which is less concave than the posterior surface, uppermost. Alternatively, in some experiments, epithelial explants were set up, as described previously. Briefly, this involved peeling the capsule and associated epithelium away from the fiber mass and pinning it in a culture dish with the cellular surface uppermost (final volume of medium, 4 ml/dish).

Recombinant human TGFβ2 (Genzyme, Cambridge, MA) was diluted in culture medium and added immediately (10 µl per dish) at a final concentration of 0.025 to 1 ng/ml, as indicated. TGFβ2, used in this study because it is a potent inducer of cataractous changes in the lens,10 is the predominant isoform in the aqueous humor.11,12 Control preparations were not treated with TGFβ. Culture medium was renewed every second day throughout the culture period, without readdition of TGFβ unless otherwise indicated. Lenses were cultured for up to 7 days and photographed daily through the anterior pole. At the end of the culture period, lenses were fixed in Carnoy’s fixative (acetic acid:ethanol, 1:3, vol/vol), embedded in paraffin, and serially sectioned for routine histology and immunolocalization of type I collagen and α-smooth muscle actin.5 Explants were monitored daily by phase-contrast microscopy for cataractous changes.5,9

The extent of lens opacification was quantified as described previously.13 Briefly, micrographs of whole lenses recorded during culture were scanned with an x-ray scanner (3CX; XRS Corporation, CA; with XRS Omni Media and PhotoShop [Adobe, San Jose, CA] software). A series of measurements were then made using image analysis software (NIH Image, ver. 1.52; National Institutes of Health, Bethesda, MD). In some micrographs, flared reflections of the light source prejudiced the assessment of the extent of opacification in certain regions (see Figs. 3A, 3B, 3C, for example). The total assessable area was therefore outlined and measured. Only micrographs in which the assessable area represented more than 75% of the total area were used. Within the assessable area, the total area of clouding and the total number of distinct intensely white opacities were also measured. An opacification index was then calculated as follows: opacification index = number of distinct opacities/proportion of assessable area with clouding. As a test of the reproducibility of this method, repeated assessments of a typical micrograph were performed. The coefficient of variation for 10 independent assessments was 8.7% (mean opacification index, 43.8).

RESULTS

Time Course of Cataractous Changes Induced by TGFβ2

Lenses from weanling or adult rats cultured with 1 ng/ml TGFβ2 and monitored daily were used to assess the time course of lens opacification (Fig. 1). Lenses from weanling rats were monitored for 5 days only, because in most lenses, the fiber mass begins to show early signs of deterioration by 6 days of culture, irrespective of treatment (see Reference 5). Lenses from adult rats, which are less susceptible to this deterioration during culture, possibly because they are protected by a much thicker capsule, were monitored for 7 days.

When cultured without TGFβ2, lenses from both weanlings and adult rats remained transparent throughout the culture period. In contrast, anterior opacities developed in lenses cultured with TGFβ2. In each case, TGFβ2-induced lens opacification began as diffuse clouding on the anterior surface of the lens. As the response progressed, these regions condensed to form distinct opacities leaving a reduced area of clouding. Accordingly, the opacification index increased with time (Fig. 1). Distinct opacities were first detected in some lenses from weanling rats on day 2. In contrast, distinct opacities did not begin to develop in lenses from adult rats until day 4. In each case, haziness was observed on the day preceding the formation of distinct opacities, with progressive opacification throughout the remainder of the culture period. At the end of the culture period—5 and 7 days of culture—lenses from weanling and adult rats, respectively, were comparable in appearance, and the corresponding opacification indices were not significantly different. At 0.025 and 0.015 ng/ml TGFβ2, as at 1 ng/ml, lenses from adult rats showed a 2-day lag in the
onset and progression of opacification compared with those from weanlings (data not shown). No sex-related differences in time of onset or severity of cataract were noted in lenses from weanling rats (data not shown).

**Comparison of Response in Lenses and Explants**

One possible explanation for the 2-day lag in the onset of opacification in adult lenses compared with weanling lenses is that, because of the thicker capsule in the former, TGFβ may take longer to traverse the adult capsule and reach the lens cells. In lens explants the cells are directly exposed to TGFβ during culture. Therefore, the following experiment was undertaken to assess the effects of the lens capsule on the responsiveness of cultured lenses to TGFβ2.

Lens epithelial explants and whole lens cultures were prepared from either weanling or adult rats and cultured with TGFβ2 (0.025–1 ng/ml) with daily monitoring. Explants and lenses were processed in parallel within each age group. The presence of capsule wrinkling and spindle-shaped cells were used as markers for cataractous change in explants, as previously described. Generalized opacification—that is, the formation of cloudy regions with or without distinct opacities—was used as the marker for cataractous change in lenses. In both lens explants and intact lenses, the onset of cataractous changes consistently occurred 2 days earlier in cultures from weanlings than in corresponding cultures from adult rats, at 2 and 4 days of culture, respectively (data not shown).

**Effect of Age of Rat on Dose Response in Cultured Lenses**

Lenses from weanling, adult, and senile rats were exposed to TGFβ2 at concentrations ranging from 0.025 to 1 ng/ml and cultured with daily monitoring. Because cataractous changes occur earlier in weanlings than in adult rats (Fig. 1), lenses from weanling and adult (or senile) rats were assessed at 5 and 7 days of culture, respectively, in these experiments.

In lenses of all ages, a dose-dependent response to TGFβ was observed. Control lenses cultured in parallel without TGFβ2 remained transparent throughout the culture period. Distinct anterior opacities developed in lenses from adult and senile rats at all concentrations of TGFβ2 tested (Fig. 2); however, distinct opacities were observed in lenses from weanling rats only at concentrations of 0.15 ng/ml and higher (Fig. 2). At 0.025 ng/ml TGFβ2, values for both adult and senile rats, although low, were significantly higher than zero (P < 0.001) but not significantly different from each other. At higher concentrations of TGFβ2, however, lenses from senile rats exhibited a much greater response to TGFβ2 than those from younger adult and weanling rats (Fig. 2; P < 0.001).

Although no distinct opacities formed in lenses from weanling rats cultured with 0.025 ng/ml TGFβ2 by day 5 of culture (as reflected in the opacification index of zero), there was evidence of a slight response to TGFβ2. The anterior surface displayed a generalized blotchy haziness. On day 5 at this concentration of TGFβ2 in lenses from senile rats, a large proportion of the lens surface showed a generalized clouding but also included some regions of condensing opacities. In each case this clouding continued to condense to form a few distinct opacities at the end of the culture period on day 7. At higher concentrations and at all ages, most of the initially cloudy areas condensed to form distinct opacities during culture. Examples of opacification in lenses from senile rats are shown in Figures 3B and 3C. At all concentrations of TGFβ2, any opacities that formed were on the anterior surface of the lens just beneath the lens capsule, whereas the fiber mass remained transparent.

Lenses that provided data for Figure 2 were also used for histologic assessment and immunolocalization of cataract markers. Results are summarized in Table 1. Qualitatively, the responses were similar at all ages investigated. Representative micrographs of sections from senile rat lenses are shown in Figure 3. For all ages examined, anterior subcapsular plaques (Fig. 3E, 3F) were usually associated with the distinct opacities. Sections of lenses from weanling rats cultured with 0.025 ng/ml TGFβ2, which exhibited only generalized cloudiness and no distinct opacities, as described earlier, commonly displayed traces of plaque formation—that is, regions of cellular multilayering in the epithelium. At all concentrations of TGFβ2 and at all ages examined, the plaques were composed of aberrant cells, including spindle-shaped cells, as reported previously for weanling lenses.5 At all ages, plaque thickness tended to increase with the concentration of TGFβ2.

Wrinkling of the lens capsule, which is another feature of subcapsular cataract and aftercataract, was sometimes observed (data not shown). This was more frequent and distinctive in lenses from weanling rats than in corresponding lenses from adult and senile rats. The lens capsule thickens as the lens grows throughout life and it is likely that the more pronounced capsule wrinkling in lenses from weanling rats is related to their thinner capsule, which is therefore more readily distorted than the thicker capsule of the older animals.

Expression of the cataract markers type I collagen and α-smooth muscle actin almost invariably accompanied opacification and associated plaque formation (Table 1 and Fig. 3), as shown previously for lenses from weanling rats.5 Representa-
tive micrographs of lens sections from senile rats showing immunoreactivity for type I collagen and \(\alpha\)-smooth muscle actin are presented in Figure 3. Note that immunoreactivity for both type I collagen and \(\alpha\)-smooth muscle actin increased in lenses cultured with 1 ng/ml TGF\(\beta\)2 compared with those cultured with 0.025 ng/ml (cf. Figs. 3H, 3K and 3I, 3L).

**TABLE 1.** Effect of TGF\(\beta\)2 Concentration on Cataractous Changes in Lenses from Rats of Various Ages: Plaque Formation and Expression of \(\alpha\)-Smooth Muscle Actin and Type I Collagen

<table>
<thead>
<tr>
<th>TGF(\beta)2 (ng/ml)</th>
<th>Weanling</th>
<th></th>
<th></th>
<th>Adult</th>
<th></th>
<th></th>
<th>Senile</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plaques</td>
<td>Coll-I</td>
<td>(\alpha)SM</td>
<td>Plaques</td>
<td>Coll-I</td>
<td>(\alpha)SM</td>
<td>Plaques</td>
<td>Coll-I</td>
</tr>
<tr>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>0.025</td>
<td>Tr</td>
<td>Tr</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>0.15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Lenses were cultured with TGF\(\beta\)2 at the concentrations indicated. TGF\(\beta\)2 was added on day 0 of culture only, as described in Figure 1. After 5 days’ culture (weanling) or 7 days’ culture (adult and senile), the lenses were fixed, embedded, and sectioned serially and assessed by hematoxylin-eosin staining for the presence of subcapsular plaques or by immunolocalization for two cataract markers, type I collagen (Coll-I) and \(\alpha\)-smooth muscle actin (\(\alpha\)SM). Plaques are subcapsular plaques. –, Not detected; Tr, trace only; +, readily detectable in at least some cells.

![Figure 3](https://example.com/fig3.png)
Effect of Repeated Exposure to TGFβ2

Lenses from weanling rats were exposed to TGFβ2 at two concentrations for various times during a 6-day culture period. Any lenses that showed signs of deterioration between 5 and 6 days of culture were discarded. Pooled data from three replicate experiments are shown in Table 2. As in previous experiments, no changes were detected in control lenses cultured in parallel.

Repeated exposure to 0.025 ng/ml TGFβ2 substantially increased the intensity of the response (Table 2). The opacification index increased, the intensity of reactivity for type 1 collagen and α-smooth muscle actin increased, and plaque formation became more pronounced. A comparable experiment was performed using TGFβ2 at 1 ng/ml. Repeated exposure to this concentration of TGFβ2 was less effective in enhancing plaque development (Table 2). At 1 ng/ml TGFβ2 there was no difference between groups B, C, and D in mean opacification index (one-way analysis of variance). However, expression of type I collagen and α-smooth muscle actin appeared to intensify with repeated doses (data not shown). Lenses that received three repeated doses of 1 ng/ml TGFβ2 (Group D; Table 2) showed development of plaques that contained exceptionally large deposits of matrix that were reactive for type I collagen (data not shown). These were even more extensive than the matrix deposits observed in senile rats (described earlier; Fig. 5F).

Effect of Reducing Time of Exposure to TGFβ2

Lenses from weanling rats were exposed to TGFβ2 for 8 to 48 hours, and then cultured without TGFβ2 for the remainder of the 5-day culture period, with the usual change of medium every 2 days. Irrespective of time of initial exposure to TGFβ2, distinct opacities developed across the anterior surface of the lens. Extending the time of TGFβ2 exposure from 8 to 24 to 48 hours did not result in a statistically significant change in the opacification index, although there was an apparent increase (Table 3).

Lenses that had been exposed to TGFβ2 for 8 or 48 hours were examined histologically. Subcapsular plaques were invariably present, but the thickness of the plaques tended to be greater with the longer exposure. Specific reactivity for type I collagen and α-smooth muscle actin was detected within and between the subcapsular plaques induced by TGFβ2 in each case (data not shown).

To assess the stability of TGFβ under culture conditions, 1 ng/ml TGFβ2 was preincubated for 8 hours. Lenses from weanling rats were introduced into this medium (time 0) then cultured for 48 hours. Medium was replaced with control medium on days 2 and 4. Lenses collected on day 5 exhibited distinct anterior opacities, and the opacification index was not significantly different from lenses cultured with fresh TGFβ2 for 48 hours (Table 3). Thus TGFβ2 retained the ability to stimulate cataractous changes beyond 8 hours in culture. Nevertheless, no significant increase in the opacification index was observed when lenses were cultured with TGFβ2 for longer periods.

Therefore, elevation of active TGFβ2 levels in the ocular media of only relatively short duration, may be sufficient to initiate substantial cataractous change in the lens, leading to opacification.

**DISCUSSION**

This study shows that TGFβ2 induces similar cataractous changes in lenses from weanling, adult, and senile rats. These include opacification due to the formation of anterior subcapsular plaques. As noted in our previous studies, these plaques contain type I collagen and α-smooth muscle actin, respectively.

### TABLE 2. Effect of Repeated Exposure to TGFβ2 on Induction of Cataractous Changes in Cultured Lenses

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure to TGFβ2 (Days)</th>
<th>0.025 ng/ml TGFβ2</th>
<th>1 ng/ml TGFβ2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OI</td>
<td>Coll-I</td>
<td>αSM</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>3 ± 1*</td>
<td>Tr</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>19 ± 1*</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>65 ± 3*</td>
<td>+</td>
</tr>
</tbody>
</table>

Lenses from weanling rats were cultured with TGFβ2, under various treatment conditions, for 6 days. Group A were cultured in control medium throughout the culture period. Group B received 2 days’ exposure to TGFβ2, which was added on day 0 but not on days 2 and 4 when the medium was changed. Group C received 4 days’ exposure to TGFβ2, which was again added to the exchange medium on day 2 but not on day 4. Group D lenses were exposed to TGFβ2 throughout the 6 day culture period. TGFβ2 was included each time the culture medium was renewed. Lenses were assessed on day 6. The opacification index (OI) was determined, and expression of type I collagen (Coll-I) and α-smooth muscle actin (αSM) was assessed by immunolocalization. OI values represent the mean ± SEM of determinations of six individual lenses. - , not detected; Tr, trace only; +, readily detectable in at least some cells.

* Significantly different from the preceding value (P < 0.005) Student’s t-test.

### TABLE 3. Effect of Varying Time of Exposure to TGFβ2 on Lens Opacification

<table>
<thead>
<tr>
<th>Length of Exposure (hr)</th>
<th>Opacification Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>24</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>48</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>48*</td>
<td>51 ± 3</td>
</tr>
</tbody>
</table>

Lenses from weanling rats were cultured with 1 ng/ml TGFβ2 for the periods indicated and then in control medium for the remainder of the 5-day culture period. TGFβ2 was added on day 0 in each case. At the end of the culture period, the opacification index was determined. Values represent the mean ± SEM of determinations of six individual lenses. Values for 8 to 48 hours’ exposure are not significantly different (one-way analysis of variance).

* TGFβ2 was preincubated for 8 hours under culture conditions before addition of lenses.
suggesting that epithelial cells have undergone a transition into a fibroblastic or myofibroblastic cell phenotype. These morphologic and molecular changes are characteristic of anterior subcapsular cataract and aftercataract in humans. They are also reminiscent of the changes that occur in other biologic systems when TGFβ is activated during wound healing.

A major finding of the present study was that, although lenses from weanling, adult, and senile rats were all susceptible to the cataractogenic influence of TGFβ, lenses from older animals were more responsive than lenses from younger animals. This result was demonstrated by their response to lower concentrations of TGFβ and also by the greater extent of the opacification that occurred in response to a given concentration of TGFβ. The increased opacification was most pronounced in the lenses from senile rats, a finding that reveals yet another important similarity between TGFβ-induced cataract in rats and cataract in humans. The present finding that rat lenses became more responsive to TGFβ with aging is consistent with the knowledge that age is the single most significant risk factor for cataract in humans. It is not clear how such increased sensitivity to TGFβ arises. However, it is noteworthy that this behavior is in contrast to the generally decreased activity of lens cells with age. For example, proliferation of lens epithelial cells in vivo rapidly decreases with aging. Furthermore, the rate of fiber differentiation in vivo decreases within the first month after birth. Similarly, studies with rat and chick epithelial explants show that the ability to undergo fiber differentiation in response to fibroblast growth factor (FGF) or insulin-like growth factor (IGF), respectively, is reduced with aging. In rats, the age-related reduction in responsiveness of lens epithelial cells to the fiber differentiating activities of FGF correlates with an age-related reduction in expression of IGF receptors in the lens. At present, there is no information on the expression of TGFβ receptors in lenses from adult rats. Immunolocalization studies have shown, however, that reactivity for type I and type II TGFβ receptors in the lens increases between the neonatal and weanling stages.

Increased sensitivity of lens cells to TGFβ could occur in two main ways. First, the level of a factor that promotes sensitivity of lens cells to TGFβ could increase with age. Alternatively, the level of a factor that desensitizes and therefore protects lens cells from TGFβ could decrease with age. Although there are no known examples of the former possibility, there is an example of the latter. Recently, we showed that estrogen can desensitize lens cells to TGFβ when administered both in vivo and in vitro. The mechanism involved is not yet understood. However, it is a particularly important finding, because it mimics a major trend shown in humans. Epidemiologic studies indicate that the prevalence of cataract in women after menopause increases much more sharply than for men of comparable age. There are also reports of a lower prevalence of some forms of cataract in women who are undergoing hormone replacement therapy. Therefore, during aging there is evidence that female hormones protect lenses from development of cataract. The parallel between the protective effects of estrogen in the TGFβ rat model and trends in epidemiologic studies raises the possibility that in humans, estrogen may provide protection against cataract by influencing a TGFβ-mediated mechanism.

Another important feature of the present study is that it gives information about the effects of different TGFβ exposure regimens. Of note, a low dose of TGFβ administered over a prolonged period can have an effect similar to a higher dose administered over a short period. For example, maintenance of a low concentration of TGFβ (25 pg/ml), which induces minimal opacification formation with a 2-day exposure, results in the formation of distinct anterior opacities when maintained over a 6-day period. These opacities are comparable to those induced by a 40-fold higher concentration of TGFβ applied for a shorter period (see Table 2). In humans, the former scenario may arise during the progression of a chronic condition that is associated with even a marginally elevated level of TGFβ. Glaucoma may conform to this model; elevated TGFβ levels in the aqueous humor of glaucoma patients have been reported, and glaucoma is also a known risk factor for cataract. The ocular inflammatory condition uveitis may mimic the latter scenario; a brief but substantial (fourfold) increase in TGFβ levels in the ocular media has been reported during the early stages of this disease in an experimental rabbit model. Uveitis is also a risk factor for cataract.

All three mammalian isoforms of TGFβ are known to be present in the ocular environment. The proteins and corresponding mRNAs have both been detected in embryonic mouse, rat, and adult human eye. TGFβ is generally synthesized as a latent complex that is activated by cleavage of the latency-associated protein to release the 25-kDa TGFβ homodimer. Activation of TGFβ can occur in a variety of conditions—for example, by proteolysis or at extremes of pH or temperature. Once activated, TGFβ may bind to cell surface receptors that promote signal transduction or, alternatively, may be bound by several different binding proteins that further modulate TGFβ bioactivity. TGFβ-binding proteins include a soluble form of the TGFβ type III receptor, 2-macroglobulin, decorin, biglycan, and certain other extracellular matrix proteins. These molecules may promote or inhibit TGFβ activity. Work in this laboratory has shown that the ocular media, particularly the vitreous, have TGFβ inhibitory properties. α-2-Macroglobulin is present in vitreous and has been shown to prevent cataractous changes induced by TGFβ in lens epithelial explants and also in whole lenses (Hales et al., unpublished data, 1996).

Although such inhibitory molecules may normally be effective in protecting lens cells from the damaging effects of any TGFβ that is present in the ocular environment, there is evidence that they can be rendered ineffective in the presence of excess active TGFβ. Anterior subcapsular cataracts, with morphologic and molecular changes consistent with those described in human studies, develop in a transgenic mouse line that overexpresses a constitutively active form of TGFβ1 in a lens-specific manner. Furthermore, subcapsular and cortical cataracts develop in adult male rats given a high dose of TGFβ2 by intravitreal injection. There is evidence of a similar phenomenon in humans. Patients who receive intravitreal TGFβ2, to promote the closure of holes in the retina exhibit an unusually high incidence of cataract.

These studies indicate that the mechanisms that normally regulate TGFβ availability to lens cells can be overcome by swamping the cells with large amounts of TGFβ. The present study, however, shows that lens cells can be extremely sensitive to the cataractogenic effects of TGFβ, indicating the exquisite control of TGFβ bioactivity that must be exercised in situ. Exposure to as little as 25 pg/ml TGFβ2 for 2 days or to 1 ng/ml for only 8 hours was sufficient to induce opacification and associated cataractous changes in the rat model. There-
fore, in the human many factors may contribute to the development of TGFβ-induced cataracts. These may include the status of the TGFβ (i.e., whether it is active or inactive due to latency or binding to inhibitory molecules), the concentration of TGFβ in the cellular microenvironment and the isoform(s) present, and the gender and hormonal status of the individual. The present study emphasizes that increased sensitivity of the lens cells to TGFβ with aging is a major factor that must also be taken into account.

In conclusion, this study further emphasizes the usefulness of TGFβ-induced cataract in rats as a model for studying human cataractogenesis. Not only did TGFβ induce lens cells from rats of the major age groups (weanling, adult, and senile) to undergo morphologic and molecular changes that mimic those of the human condition, but also the lens cells showed increased susceptibility to the cataractogenic effects of TGFβ with age. These results emphasize the need for greater understanding not only of how TGFβ bioactivity is regulated, but also of the factors that modulate the responsiveness of lens cells to TGFβ and how these aspects of lens biology are influenced by aging. The results suggest that at least some forms of cataract may arise from an elevation in the level of TGFβ activity, of either short or long duration, or from an age-related increase in lens cell responsiveness to TGFβ. The etiology of cataract during aging or otherwise, however, is likely to be multifactorial, including both the direct effects of TGFβ on lens cells and/or as yet undetermined cellular influences related to other known risk factors.

Acknowledgments

The authors thank Cedric Shorey and Arthur Everitt, Department of Anatomy and Histology, The University of Sydney, for the generous donation of senile rats, which came from an aged colony housed at Concord Repatriation Hospital, Sydney, and Roland Smith for assistance with photography.

References


