Cataract Induction in Lenses Cultured With Transforming Growth Factor-β

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

Purpose. Anterior subcapsular cataracts are characterized by the appearance of opaque plaques of abnormal cells. Distinctive spindle-shaped cells containing α-smooth muscle actin are present and are associated with wrinkling of the overlying lens capsule. Accumulations of extracellular matrix, including type I collagen, also are found. The authors previously reported that transforming growth factor-β (TGF-β) induces similar aberrant morphologic changes in lens epithelial explants. More recently, they identified α-smooth muscle actin in explants cultured with TGF-β. The aim of this study was to determine whether TGF-β induces comparable cataractous changes in whole lenses and to examine the effects of this treatment on the transparency of the lens.

Methods. Whole lenses from 21-day-old rats were cultured in defined serum-free medium with TGF-β2 or without added growth factors for 5 days. Lenses were then photographed and prepared for histology and immunolocalization.

Results. Lenses cultured with TGF-β developed distinct anterior opacities just beneath the lens capsule. Histologically, clumps of abnormal cells corresponded with these opacities. Spindle-shaped cells, which contained α-smooth muscle actin, were present, and the overlying capsule was often wrinkled. The clumps contained accumulations of type I collagen, laminin, and heparan sulphate proteoglycan. In contrast, lenses cultured without growth factors remained transparent, retained normal lens morphology, and did not accumulate α-smooth muscle actin or type I collagen.

Conclusions. These results show that TGF-β induces whole lenses to form opacities that contain morphologic and biochemical markers for subcapsular cataract.

Cataract, an opacity of the lens that may cause partial or total blindness, is one of the most prevalent eye diseases. Extracapsular cataract extraction, the most common treatment in Western countries, is not always successful; in 10% to 50% of patients, reclouding of the lens occurs within 5 years because of the formation of after-cataracts (also referred to as posterior capsular opacifications or secondary cataracts) that arise from lens cells left behind at the time of surgery.1-3

Given the magnitude of the cataract problem worldwide (for example, 1.3 million cataract operations costing $5 billion were performed in the United States in 1991),4 surprisingly little is known about its cause(s). Epidemiologic studies have identified numerous factors associated with increased risk of cataract (for example, aging, diabetes, malnutrition, ultraviolet light—sunlight, glaucoma, and ocular surgery),5 and a protective role for antioxidant nutrients, vitamins, and minerals has been suggested but not proven.6 However, little is known about mechanisms of cataractogenesis at the molecular or cellular level; this has hampered progress in clinical management.

Previously, using rat lens epithelial explants, we showed that fibroblast growth factor plays a key role in normal events in mammalian lens growth and differentiation.7,8 We reported recently9 that, in contrast, transforming growth factor-β (TGF-β), a multifunctional growth factor, induces abnormal changes in explants, all of which can be blocked by a pan-specific

From the Department of Anatomy and Histology, University of Sydney, Sydney, New South Wales, Australia.
Supported by grants from the National Eye Institute, the Department of Health, Education and Welfare, and the National Health and Medical Research Council, Canberra, Australia, and by a University of Sydney Medical Faculty Postgraduate Scholarship (AMH).
Submitted for publication November 28, 1994; revised March 13, 1995; accepted March 13, 1995.
Proprietary interest category: N.
Reprint requests: John W. McAvoy, Department of Anatomy and Histology, University of Sydney, Sydney, New South Wales, Australia 2006.
antibody against TGF-β. These include the formation of spindle-shaped cells, capsule wrinkling, and accumulation of extracellular matrix (ECM). Some or all of these changes are typically found in human subcapsular cataracts and after-cataracts. The lens explants we use consist of epithelial cell monolayers peeled away from fiber cells (see Fig. 1C for normal lens morphology) and pinned out on culture dishes. Although this culture system is well suited to analyzing responses to TGF-β at the cellular level, it does not allow assessment of the effects of TGF-β on the most clinically significant feature of cataract induction, opacification of the lens. In this article, we show that whole rat lenses cultured with TGF-β develop opacities that closely resemble anterior subcapsular cataract.

MATERIALS AND METHODS

All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty-one-day-old rats were sacrificed by carbon dioxide asphyxiation, and eyes were removed and placed in approximately 4 ml serum-free culture medium (M199 with bovine serum albumin and antibiotics). Lenses were carefully removed by a posterior approach using plastic-coated forceps and fine scissors and were transferred to a culture dish containing 3 ml fresh medium (two lenses per dish). Transforming growth factor-β2 (Genzyme, Cambridge, MA) was added to the culture medium at a final concentration of 5 ng/ml; this was shown to be a saturating dose in studies using lens explants from 21-day-old rats (Liu J, Chamberlain C, McAvoy J, unpublished data, 1993). Control lenses were cultured without TGF-β. Lenses (six per treatment) were cultured for 5 days at 37°C in 5% CO2/air. Medium (with or without TGF-β) was renewed on days 2 and 4. Lenses were photographed daily and fixed and embedded for routine histology on day 5. Results were confirmed in a replicate experiment. In supplementary experiments, lenses were cultured for longer periods. Beyond 5 days culture, there was no further progression of TGF-β-induced changes; beyond 7 days, irrespective of treatment, deterioration of the lens fiber mass was observed.

At the end of the culture period, whole lenses were fixed overnight in Carnoy’s fixative, dehydrated in alcohol, cleared in xylol at room temperature, and embedded in paraffin. Serial 3-μm sections were dried onto slides overnight at 37°C and stained with hematoxylin and eosin. Alternatively, immunofluorescent localization was performed using antibodies against α-smooth muscle actin (clone IA4; Sigma, St. Louis, MO), laminin, heparan sulphate proteoglycan (HSPG), or rat type I collagen (Silenus, Hawthorn, Vic, Australia). Further details of localization methods are given elsewhere. Appropriate nonimmune immunoglobulin or serum controls were included routinely. For collagen type I, predesorption controls were also carried out. The specificity of the α-smooth muscle actin antibody has been established by Western blot analysis.

RESULTS

When lenses from 21-day-old rats were maintained in medium without TGF-β (controls), lenses remained transparent (Fig. 1A) and retained normal morphology during the 5-day culture period (Figs. 1C, 2A). When TGF-β was included in the medium, however,
TGF-β Induces Anterior Subcapsular Cataracts In Vitro

FIGURE 2. Micrographs of lenses cultured without transforming growth factor (TGF)-β (A,C) or with TGF-β (B,C) for 5 days. Serial sections were stained with hematoxylin and eosin (A,B) or used for immunofluorescent localization of α-smooth muscle actin (C,D). Control lenses retained normal lens architecture with a monolayer of cuboidal epithelium overlying the fiber mass (A); no reactivity for α-smooth muscle actin was detectable (C). Subcapsular plaques containing spindle-shaped cells (closed arrow) developed in lenses cultured with TGF-β, and between the plaques (open arrow), abnormal cells replaced the cuboidal epithelium (B). Insets show the region indicated by the open arrow in B and the corresponding region in A at higher magnification. Spindle-shaped cells within and outside the plaques showed strong reactivity for α-smooth muscle actin (D). Scale bar = 30 μm (insets, 20 μm).

distinct subcapsular opacities formed across the anterior surface of every lens (Fig. 1B), whereas the fiber cells that comprised the bulk of the lens remained transparent. The opacities, first noticed at 3 days, became larger and more opaque with time. They appeared to correspond with subcapsular clumps or “plaques” of aberrant cells distributed across the anterior surface of the lens (Figs. 1D, 2B). Between the plaques, spindle-shaped cells were arranged parallel to the capsule; no normal cuboidal epithelial cells remained (Figs. 2A, 2B). The spindle-shaped cells were generally present in multilayers two to five cells deep, and the thickest regions tended to be associated with wrinkling of the overlying capsule (Fig. 3). There also were regions devoid of these cells, in which the capsule abutted directly onto the fiber mass. Some of the abnormal cells in the subcapsular plaques also appeared to be spindle shaped (Fig. 2B).

Lenses cultured with TGF-β showed strong reactivity for α-smooth muscle actin within and outside the plaques (Fig. 2D) in regions in which spindle-shaped cells were present. Corresponding sections treated with normal mouse immunoglobulin G instead of the α-smooth muscle actin primary antibody (to test for nonspecific fluorescence) showed no reactivity for α-smooth muscle actin (not shown). No reactivity for α-smooth muscle actin was detected in control lenses (Fig. 2C).

In lenses cultured with TGF-β, the subcapsular plaques were shown to contain accumulations of laminin and HSPG (Figs. 4B, 4D), ECM components normally present in lens capsule.19 Strong reactivity for laminin was present throughout the plaques, but HSPG reactivity was concentrated in the central regions (Figs. 4B, 4D). In control lenses, laminin and

FIGURE 3. Micrograph of a lens cultured with transforming growth factor-β for 5 days and stained with hematoxylin and eosin, showing wrinkling of the capsule (ca) and spindle-shaped cells (arrowhead). Scale bar = 17 μm.

FIGURE 4. Micrographs of lenses cultured without transforming growth factor (TGF)-β (A,C,E) and with TGF-β (B,D,F). Serial sections were used for immunofluorescent localization of extracellular matrix components: laminin (A,B), heparan sulphate proteoglycan (C,D), or type I collagen (E,F). Control lenses displayed a normal distribution of laminin (A) and heparan sulphate proteoglycan (HSPG) (C) in the lens capsule and showed no reactivity for type I collagen (E). Lenses cultured with TGF-β showed strong reactivity for laminin (B) and HSPG (D) in the subcapsular plaques and the lens capsule. Reactivity for type I collagen was present within these plaques and in association with the spindle-shaped cells that replaced the normal epithelium (F). Scale bar = 30 μm.
HSPG were detected in the capsule only (Figs. 4A, 4C). In addition, type I collagen, which is not generally found in the lens, was detected within the plaques and in association with spindle-shaped cells outside the plaques (Fig. 4F). In control lenses, no specific fluorescence for type I collagen was detected (Fig. 4E). Corresponding sections treated with normal rabbit immunoglobulin G instead of any of the specific antibodies, to test for nonspecific fluorescence, showed no reactivity either in the plaques or in the lens capsule. For type I collagen, specific reactivity was virtually abolished by preadsorbing the antibody with type I collagen (not shown).

**DISCUSSION**

In this study, we identified a molecule present in the eye that induces cataractous changes in the lens in vitro. Subcapsular opacities histologically indistinguishable from early stages of human anterior subcapsular cataracts develop in whole rat lenses cultured with TGF-β2 for 5 days. This form of cataract is seen first as small, opaque flecks or dots underlying the capsule and overlying the transparent fiber mass. Histologically, these opacities consist of plaques of abnormal cells, including spindle-shaped cells, and accumulations of ECM; the capsule overlying the plaques is often wrinkled. Type I collagen and α-smooth muscle actin, proteins not normally found in the lens, also are found in this form of cataract.

In the opaque subcapsular plaques induced by TGF-β, abnormal accumulations of laminin and HSPG were present (Figs. 4A to 4D; see ref. 10); these are normal lens capsule components. Type I collagen also accumulates in these plaques (Fig. 4F). The latter finding is supported by an earlier study in which type I collagen was localized in TGF-β-induced cellular aggregations in postnatal rat lens explants (Liu J, Chamberlain C, McAvoy J, unpublished data, 1994). Type I collagen is an ECM component known to accumulate in anterior subcapsular cataracts and after-cataracts. It is not usually expressed in the lens, although it has been detected in the capsule of some elderly patients. Thus, TGF-β induces cataract-like accumulation of ECM components, including a marker for cataract. The cytoskeletal protein α-smooth muscle actin has been localized in the spindle-shaped cells of anterior subcapsular cataracts and after-cataracts; however, it is not present in the normal mammalian lens. Recently, we showed that this marker for cataract is induced in lens explants by TGF-β. In this article, we show that α-smooth muscle actin is present in the spindle-shaped cells and cataract-like subcapsular plaques induced in whole lenses by TGF-β (Figs. 2C, 2D).

Transforming growth factor-β and its mRNA have been detected in the mammalian eye. Furthermore, several studies have shown that the ocular media that bathe the lens contain TGF-β, and they suggest that, under normal conditions, this TGF-β is present predominantly in a latent form. A major increase in vitreous TGF-β levels has been detected in proliferative vitreoretinopathy, and unusually high levels of active TGF-β have been reported in another study in which aqueous was collected only from patients with cataract. In addition, latent TGF-β may become activated—for example, after eye surgery.

In summary, this study of whole lenses has identified a molecule capable of inducing lens opacification and early events in cataractogenesis. The opacities induced by TGF-β are indistinguishable from classic textbook representations of early stages of anterior subcapsular cataract, and they contain two molecules known to be markers for this form of cataract and for after-cataract. Moreover, this study has established that, at least in vitro, lens cells can receive a TGF-β stimulus across the intact lens capsule, which must happen if elevations in ocular media TGF-β activity are involved in the induction of anterior subcapsular cataract in situ. All these findings are consistent with a key role for TGF-β in the etiology of major forms of cataract.

**Key Words**

α-smooth muscle actin, cataractogenesis, lens opacities, transforming growth factor-β, type I collagen

**Acknowledgments**

The authors thank Dr. F. Lovicu for assistance with the type I collagen preadsorption control. The authors also thank R. Smith for assisting with photography and Dr. M. Dziadek for supplying the heparan sulphate proteoglycan antibody.

**References**

TGF-β Induces Anterior Subcapsular Cataracts In Vitro


