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# Trabecular Cells of the Eye Express Messenger RNA for Transforming Growth Factor- $\beta$ 1 and Secrete This Cytokine

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**Purpose.** To determine whether trabecular tissue in vivo and cultured trabecular cells have the messenger RNA transcript for transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), and to examine whether these cells synthesize and secrete TGF- $\beta$ 1 in vitro.

**Methods.** Total RNA was isolated from the trabecular meshwork, iris, and ciliary body freshly excised from porcine eyes as well as from cultured trabecular cells, and the reverse transcriptase-polymerase chain reaction and Southern hybridization were used for detection of TGF- $\beta$ 1 messenger RNA. The amount of TGF- $\beta$ 1 secreted by trabecular cells in culture was determined by radioimmunoassay.

**Results.** Excised whole trabecular tissue, iris, and ciliary body, as well as cultured trabecular cells expressed messenger RNA transcripts for TGF- $\beta$ 1. On the ethidium bromide-stained agarose gel, two PCR-amplified products (161 and 400 base pairs) were found in the total RNA isolated from cultured trabecular cells. The oligonucleotide probe specific for TGF- $\beta$ 1 detected only one band with the expected length of 161 base pairs. The secretion of TGF- $\beta$ 1 into conditioned medium was at the level of 16.7–20 pg/ml per 2 million trabecular cells during a 24-hr period.

**Conclusions.** These investigations show that the trabecular meshwork, iris, and ciliary body in vivo express the messenger RNA transcript for TGF- $\beta$ 1, and that trabecular cells in vitro synthesize and secrete this cytokine. The TGF- $\beta$ 1 present in normal aqueous humor may be derived locally, at least in part, from the cells of the trabecular meshwork, iris, and ciliary body. Abnormal synthesis, secretion, activation, and clearance of TGF- $\beta$ 1 may contribute to the pathogenesis of many ocular disorders, including primary open-angle glaucoma. Invest Ophthalmol Vis Sci. 1993;34:2562–2569.

The transforming growth factor (TGF)- $\beta$ s are a family of regulatory proteins that make up at least six closely related isoforms with 64% to 82% sequence homology. These polypeptides are usually homodimeric, with an apparent molecular weight of approximately 25 kDa.<sup>1-3</sup> They exhibit pleiotropic activities on a broad spectrum of cellular targets.<sup>1,4</sup> Significant

amounts of TGF- $\beta$ 1 and - $\beta$ 2 are detectable in the normal aqueous humor of several different mammalian species, including humans, rabbits, cows, mice, and pigs.<sup>5-8</sup> However, the origins and cellular sources of synthesis of TGF- $\beta$ 1 in the aqueous humor are not completely understood.<sup>8</sup> Previous investigations have shown that murine iris and ciliary body cells as well as epithelial cells, endothelial cells, and stromal fibroblasts of the human cornea produce the messenger ribonucleic acid (mRNA) for TGF- $\beta$ 1<sup>9-11</sup> and synthesize this cytokine,<sup>9</sup> which may partly contribute to its presence in the aqueous humor.

Recently, we identified, partially characterized,<sup>12</sup> and quantitated<sup>13</sup> TGF- $\beta$ 1 receptors on porcine tra-

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trabecular cells, and we determined that exogenous TGF- $\beta$ 1 can modulate the growth and function of trabecular cells in culture.<sup>12</sup> Our purpose in the current study was to investigate whether trabecular cells express mRNA transcripts for TGF- $\beta$ 1 and produce and secrete this cytokine.

## MATERIALS AND METHODS

All chemicals, enzymes, and reagents used in the experiment were purchased from Promega (Madison, WI) unless otherwise specified.

### Tissue Preparation of Trabecular Meshwork, Iris, and Ciliary Body

Cultures of trabecular cells were initiated from normal porcine eyes obtained from a local abattoir within 20 min of decapitation, as described previously.<sup>12,14,15</sup> The use of animal tissue in this investigation adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Briefly, the eyes were opened by a circumferential incision at the ora serrata. The anterior segment was placed in a sterile petri dish with its external surface down, and, after being severed from its zonular attachments, the lens was removed. The anterior segment was then divided into two halves with a sharp razor blade. Under a dissecting microscope, the iris was retracted carefully from the inner corneal surface, and the pectinate ligaments were cut with microscissors at their insertion into the periphery of the cornea and the uveal meshwork. The iris and ciliary body were excised, frozen immediately on dry ice, and stored at  $-82^{\circ}\text{C}$  until analyzed. The trabecular meshwork in the internal corneoscleral sulcus was exposed, and two parallel partial-thickness incisions were made with a Beaver microsurgical knife, one posterior to the periphery of the cornea and the other anterior to the ciliary musculature. The tissue of trabecular meshwork was lifted gently with fine jeweler's forceps and placed in tissue culture medium (Eagle's minimal essential medium supplemented with 15% newborn calf serum). Explants of trabecular tissue 3–4 mm wide were placed in 100 mm  $\times$  25 mm culture dishes and incubated at  $37^{\circ}\text{C}$  in culture medium. All experiments with trabecular cells were performed on primary cultures after 3–4 weeks of incubation, similar to those described previously.<sup>12</sup> For analysis of whole trabecular tissue, several samples were frozen on dry ice and stored at  $-82^{\circ}\text{C}$ .

### Extraction of Total RNA

Total RNA was extracted from whole tissue samples or cultured cells according to the method described by Chomczynski and Sacchi.<sup>16</sup> Briefly, 0.6 ml of denaturing solution (guanidine thiocyanate dissolved in citrate/sarcosine/ $\beta$ -mercaptoethanol buffer) was dispensed into a sterile dounce glass-Teflon homogenizer

(Kontes Glass Co., Vineland, NJ) and chilled on ice for 5 min. Fifty milligrams of wet weight trabecular tissue or fifty milligrams of wet weight iris and ciliary body was placed into the denaturing solution, minced, and disrupted by the homogenizer. After adding 0.06 ml of 2 M sodium acetate at pH 4.0, the solution was mixed thoroughly by vortexing and then 0.6 ml of phenol:chloroform:isoamyl alcohol was added. This solution was mixed, chilled on ice for 15 min, and subjected to centrifugation at  $10,000 \times g$  for 20 min at  $4^{\circ}\text{C}$ . The aqueous phase was transferred to another tube, an equal volume of isopropanol was added, and the mixture was incubated overnight at  $-82^{\circ}\text{C}$ . The RNA was pelleted by centrifugation at  $10,000 \times g$  for 15 min at  $4^{\circ}\text{C}$  and resuspended in 0.25 ml of denaturing solution. After the RNA was dissolved, 0.25 ml of isopropanol was added and the RNA was precipitated overnight at  $-82^{\circ}\text{C}$ . The RNA was collected, washed with 1 ml of ice-cold 75% ethanol, and dried in a Speed Vac system (Savant Instruments, Inc., Farmingdale, NY). After resuspension in 40  $\mu\text{l}$  of ribonuclease-free water, we determined the amount of RNA by using a Spectronic 21 UVD spectrophotometer (Milton Roy Company, Rochester, NY) at a wavelength of 260 nm.

For extraction of RNA from trabecular cells, the cells were washed three times with ice-cold phosphate buffered saline. The denaturing solution was then added to the culture dish, and cell lysis was confirmed with an optical microscope. Next, the solubilized cells were aspirated and transferred into a dounce glass-Teflon homogenizer. Thereafter, processing of the cells was similar to that described for the tissue samples.

### Synthesis of First-Strand Complementary DNA

First-strand complementary DNA (cDNA) was prepared from total tissue or cellular RNA by using avian myeloblastosis virus reverse transcriptase and the downstream primer specific for TGF- $\beta$ 1 (Clontech Laboratories, Inc., Palo Alto, CA). The reverse transcription reaction was performed in a final volume of 20  $\mu\text{l}$  with 5 mmol/l of magnesium chloride ( $\text{MgCl}_2$ ), 10 mmol/l tris hydrochloride, 50 mmol/l potassium chloride, 0.1% Triton X-100, 1 mmol/l each deoxyribonucleotides (deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxythymidine triphosphate, and deoxyguanosine triphosphate), 20 units of rRNasin ribonuclease inhibitor, 15 units of avian myeloblastosis virus reverse transcriptase, 0.1  $\mu\text{M}$  of downstream primer, and 5  $\mu\text{g}$  of total RNA. The reaction was allowed to proceed at  $42^{\circ}\text{C}$  for 60 min, heated at  $99^{\circ}\text{C}$  for 5 min, then incubated at  $3^{\circ}\text{C}$  for 5 min.

### Amplification of cDNA by Polymerase Chain Reaction

The polymerase chain reaction (PCR) primers specific for TGF- $\beta$ 1 were purchased from Clontech, and their sequences from 5' to 3' were as follows: upstream

primer, GCCCTGGACACCAACTATTGCT, downstream primer, AGGCTCCAAATGTAGGGGCGAGG, with an expected size of the amplified sequence of 161 base pairs.<sup>17,18</sup> The primer pair spans one intron<sup>19,20</sup> which contains about 557 nucleotides;<sup>21</sup> thus, the amplification of contaminating genomic DNA sequence would yield a PCR product larger than that amplified from cDNA. We diluted the products from the reverse-transcription reaction to 97.5  $\mu$ l and used 20  $\mu$ l to perform the PCR amplification in a total volume of 100  $\mu$ l with 2.5 units of native *Taq* DNA polymerase (Perkin-Elmer Corp., Norwalk, CT), 1.5 mmol/l  $MgCl_2$ , and 1.0  $\mu$ M primers. We used a DNA thermal cycler 480 (Perkin-Elmer Cetus) with the temperature profiles as follows: initial melting at 94°C for 4 min, then 30 cycles of 1 min melting at 94°C, 2 min annealing at 60°C, and 3 min extension at 72°C. After the last cycle, the polymerization step was extended by 10 min more so that all strands were completed. We also performed simultaneous experiments by using positive-control DNA template for TGF- $\beta$ 1 and negative-control reactions without template.

#### Southern Blotting of PCR Products

Horizontal 2% agarose gel electrophoresis with 8  $\mu$ l of PCR products, 2  $\mu$ l of 5 $\times$  loading buffer, and 1  $\mu$ l of 1 mg/ml ethidium bromide was performed in a mini-submarine GNA-100 gel apparatus (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) at 50 V for 3 hr. Phi X 174 DNA/*Hae* III digests were used as molecular size standards. The separated DNA products were transferred to a 0.2  $\mu$ m pore nitrocellulose membrane (Schleicher and Schuell, Keene, NH) with 0.4 N NaOH, 0.6 N NaCl solution by means of a vacuum transfer apparatus (Hoefer Scientific Instruments, San Francisco, CA) for 1 hr and then fixed to the membrane in an ultraviolet crosslinker (Hoefer Scientific Instruments).

#### Hybridization of TGF- $\beta$ 1 Probe to Southern Blot

Southern hybridization with 30-mer TGF- $\beta$ 1 antisense oligonucleotide probe (Clontech Laboratories, Inc.) that hybridizes to regions within the amplified sequences was performed. We used bacteriophage T4 polynucleotide kinase (Clontech Laboratories, Inc.) to label the 5' end of the probe (100 ng) with [ $\gamma$ -<sup>32</sup>P]-adenosine triphosphate (>7,000 Ci/mmol, ICN Biomedicals, Inc., Irvine, CA). Unincorporated nucleotides were removed from the labeled probe by centrifugation on a Chroma spin-30 column centrifuge (Clontech Laboratories, Inc.). The specific activity of the probe was determined in a scintillation counter (Packard Instrument Company, Meriden, CT), and the solution was diluted to a final concentration of  $4 \times 10^6$  cpm/ml with hybridization solution (5 $\times$  sodium chloride/sodium citrate (SSC), 1 $\times$  Denhardt's solution, and 100  $\mu$ g/ml denatured salmon testes DNA). We

prehybridized the southern blot at 55°C in prehybridization solution (5 $\times$  SSC, 5 $\times$  Denhardt's solution, and 100  $\mu$ g/ml denatured salmon testes DNA) in an incubator (Robbins Scientific Corp., Sunnyvale, CA) for 3 hr, and then with the labeled probe in hybridization solution for 20 hr at 55°C. After hybridization, the blot was rinsed in 2 $\times$  SSC, 0.05% sodium dodecyl sulfate at room temperature for 30 min and then washed with the same solution at 55°C for 20 min. We performed autoradiography by using an intensifying screen for 2–5 hr at room temperature with OMAT-AR film (Eastman Kodak Co., Rochester, NY).

#### Preparation of Conditioned Medium from Trabecular Cells

Conditioned medium was prepared by a modification of a previously described method.<sup>22</sup> Briefly, trabecular cells grown to confluence (about 2,000,000 cells) in 15% newborn calf serum supplemented culture medium were washed twice with phosphate-buffered saline, and then with serum-free medium three times each for 10 min. To eliminate carry-over of TGF- $\beta$ 1 from serum, we added serum-free medium alone to the cultures with 3 changes of medium at 8-hr intervals for a 24-hr period. Thereafter, 3 ml of fresh serum-free medium was added to each dish, allowed to condition for 24 hr, and then collected. The latter procedure was repeated for four consecutive days. All conditioned media, including those from the last two 8-hr washings, were centrifugated at 500  $\times$  g to remove cellular debris, and 1  $\mu$ g/ml aprotinin, leupeptin, and pepstatin A were added. The media were then concentrated to a final volume of 500  $\mu$ l by ultrafiltration with Amicon 10 kD cutoff filters (Amicon Corp., Danvers, MA). All conditioned media were assayed with and without acid activation. For acid activation, the samples were treated with 2 N HCl at pH 2 for 30 min. After neutralization with 1 N NaOH or with the buffer (0.1 M tris hydrochloride, pH 8.0, 4% bovine serum albumin, 1% NP-40, 0.2% sodium dodecyl sulfate, 0.15 M NaCl), the samples were assayed as described below.

#### Radioimmunoassay for TGF- $\beta$ 1 in Trabecular Cell-Conditioned Medium

A two-step binding assay that involved the delayed addition of labeled TGF- $\beta$ 1 was used.<sup>8,22</sup> One hundred microliters of TGF- $\beta$ 1 standard or of the activated or nonactivated samples of conditioned medium was mixed with 200  $\mu$ l of assay buffer and preincubated with 100  $\mu$ l of polyclonal chicken anti-TGF- $\beta$ 1 neutralizing antibody (25  $\mu$ g/ml) or polyclonal chicken control antibody (Oncomembrane Inc., Seattle, WA) for 24 hr at room temperature. Next, 100  $\mu$ l of <sup>125</sup>I-TGF- $\beta$ 1 (20,000 cpm; Dupont Co., Boston, MA) was added, and the mixture was incubated at room temperature for an additional 24 hr. Then 100  $\mu$ l of affinity puri-

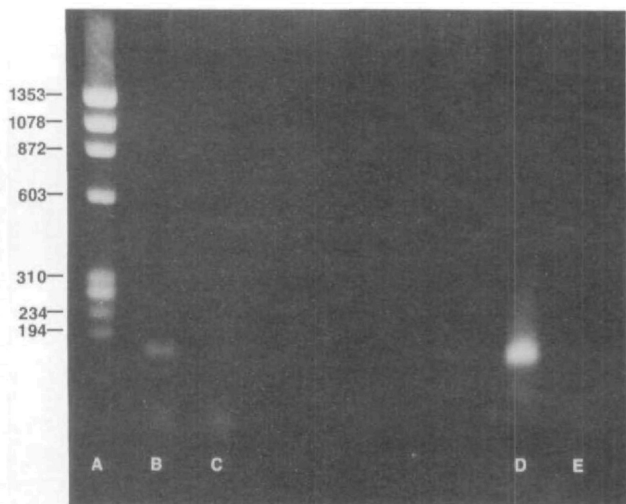
fied rabbit anti-chicken (H+L) immunoglobulin G (100  $\mu$ g/ml; Jackson Immunoresearch, Pittsburgh, PA) was added, and the mixture was allowed to stand for 2 hr at room temperature. After centrifugation at 2200  $\times$  g at 4°C for 30 min, the supernatant was aspirated, and the radioactivity of the precipitate was counted in a mini-gamma counter (Pharmacia LKB Nuclear Inc., Gaithersburg, MD). All experiments were performed in triplicate.

**RESULTS**

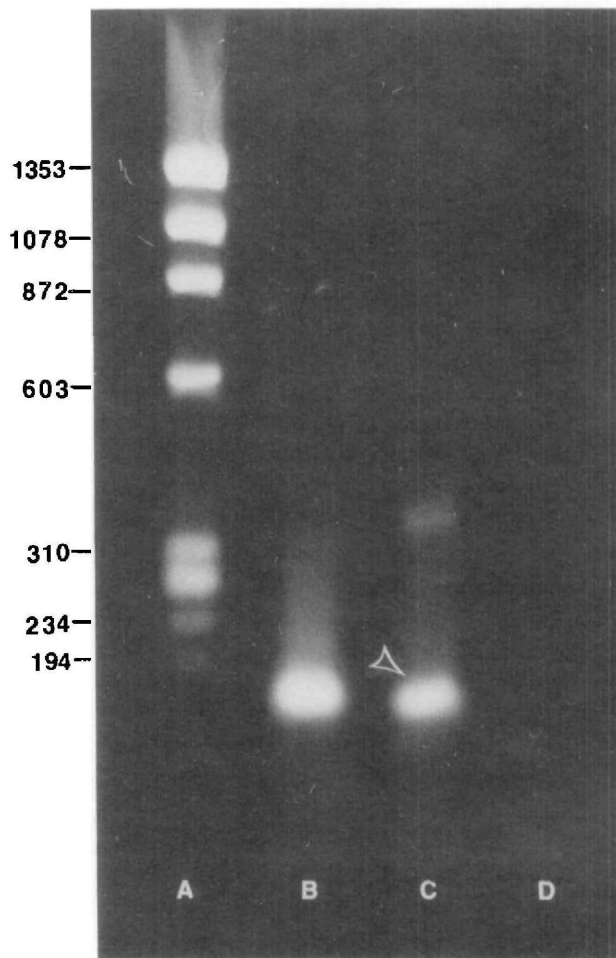
**Extraction of Total RNA and PCR Amplification**

Approximately 30  $\mu$ g of RNA was recovered from 50 mg of whole trabecular tissue excised from 8 porcine eyes, 45  $\mu$ g of RNA from 50 mg of iris and ciliary body tissue, and 20  $\mu$ g of RNA from  $1.6 \times 10^6$  trabecular cells. All of the RNA was substantially free of DNA and contaminating protein, exhibiting  $A_{260}/A_{280}$  ratios that ranged from 1.71 to 1.92.

By using primers specific for TGF- $\beta$ 1, we obtained a PCR-amplified product that corresponded to 161 base pairs from whole trabecular tissue, iris, and ciliary body, as well as from the positive control TGF- $\beta$ 1 DNA template (Fig. 1). The size of amplified fragments from trabecular meshwork, iris, and ciliary body tissues was determined by regression analysis with the Phi X 174 DNA/*Hae* III standard size markers, and by comparison with the product from the positive control template. In cultured trabecular cells, we detected a major band that corresponded to 161 base pairs and a fainter band at the 400-base-pair position (Fig. 2). The 161-



**FIGURE 1.** PCR products of TGF- $\beta$ 1 amplified from tissue of the iris and ciliary body (*lane B*), tissue of trabecular meshwork (*lane C*), positive control DNA template (*lane D*), and negative control (*lane E*). Phi X 174 DNA/*Hae* III standard size markers are shown in *lane A*, and their lengths in base pairs are indicated on the left.



**FIGURE 2.** PCR products of TGF- $\beta$ 1 amplified from positive control DNA template (*lane B*), cultured trabecular cells (*lane C*), and negative control (*lane D*). The arrow marks the expected 161-base-pair band. DNA standard size markers are shown in *lane A*, and their lengths are indicated on the left.

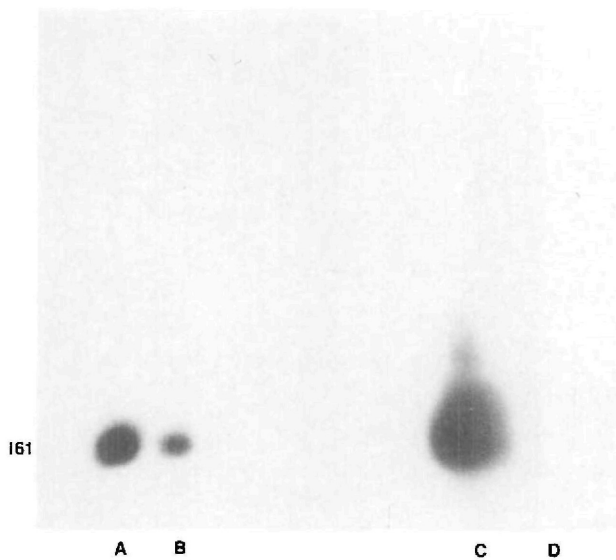
base-pair PCR product comigrated with the sequence amplified from TGF- $\beta$ 1 positive-control template.

**Hybridization of TGF- $\beta$ 1 Probe to Southern Blot**

On Southern blots, the antisense oligonucleotide probe for TGF- $\beta$ 1 hybridized with the 161-base-pair PCR product obtained from excised iris, ciliary body, trabecular meshwork, as well as from cultured trabecular cells (Figs. 3, 4). The 400-base-pair reaction product amplified from trabecular cells did not hybridize with the specific probe for TGF- $\beta$ 1 (Fig. 4).

**Radioimmunoassay for TGF- $\beta$ 1 in Trabecular Cell-Conditioned Medium**

The standard radioimmunoassay calibration curve demonstrated that the sensitivity of the assay was 0.3 ng/ml (Fig. 5). The intra- and inter-assay coefficients of variation were 11.1% and 12.4%, respectively. For



**FIGURE 3.** Southern hybridization of PCR-amplified products (from Fig. 1) with TGF- $\beta$ 1 antisense oligonucleotide probe specific for the sequence within the amplified segment. *Lane A* is the product from the tissue of iris and ciliary body, *lane B* from the tissue of trabecular meshwork, *lane C* from positive control DNA template, and *lane D* from the negative control. The length in base pairs corresponding to the DNA standard size marker is indicated on the left.

the range of amounts of TGF- $\beta$ 1 analyzed, the recoveries of spiked TGF- $\beta$  in the conditioned media were quantitative and without significant inhibition for the determination of the spiked TGF- $\beta$ 1. Half-maximal inhibition of  $^{125}$ I-TGF- $\beta$ 1 was achieved at 2.3 ng/ml. The antibody used in the radioimmunoassay showed <6% cross-reactivity with TGF- $\beta$ 2 at concentrations exceeding 200 ng/ml, and it did not react with insulin-like growth factor-I, transforming growth factor- $\alpha$ , basic fibroblast growth factor, transferrin, or mouse or human epidermal growth factor. Nonspecific binding for TGF- $\beta$ 1 in the radioimmunoassay was less than 3.4%.

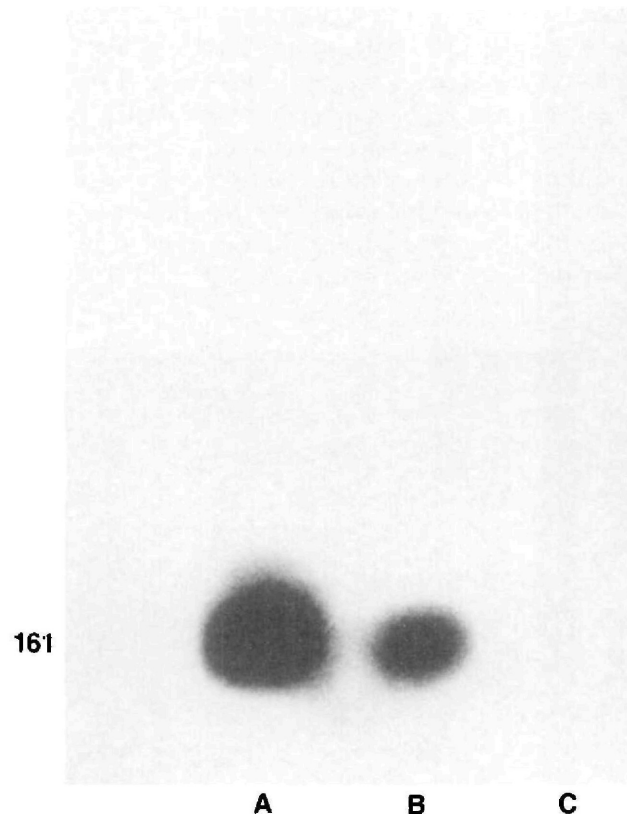
The concentrations of TGF- $\beta$ 1 in the culture medium conditioned by trabecular cells for different periods are shown in Fig. 6. TGF- $\beta$ 1 was detected at a level of  $16.7 \pm 1.85$  pg/ml only at the end of the second 24-hr incubation. This level increased to  $20 \pm 2.5$  pg/ml after the third 24-hr incubation. By the fourth day, TGF- $\beta$ 1 in the conditioned medium was undetectable, and corresponded to the cell of death. Without acid activation, no TGF- $\beta$ 1 was detected in any samples of the conditioned medium.

## DISCUSSION

By using the technique of PCR, we demonstrated that messenger RNA transcripts that encode TGF- $\beta$ 1 are present in freshly excised trabecular tissue as well as in cultured trabecular cells. By comparison to the standard molecular size markers, we obtained a PCR-am-

plified product of the expected size from cultured trabecular cells as well as from whole trabecular meshwork, iris, and ciliary body. The comigration of this product with the sequence amplified from the TGF- $\beta$ 1-positive control template implied that it was specific for TGF- $\beta$ 1. This result was confirmed by Southern blotting of the PCR product and hybridization with a specific antisense oligonucleotide probe that recognizes a unique nonprimer sequence situated within the amplified product.

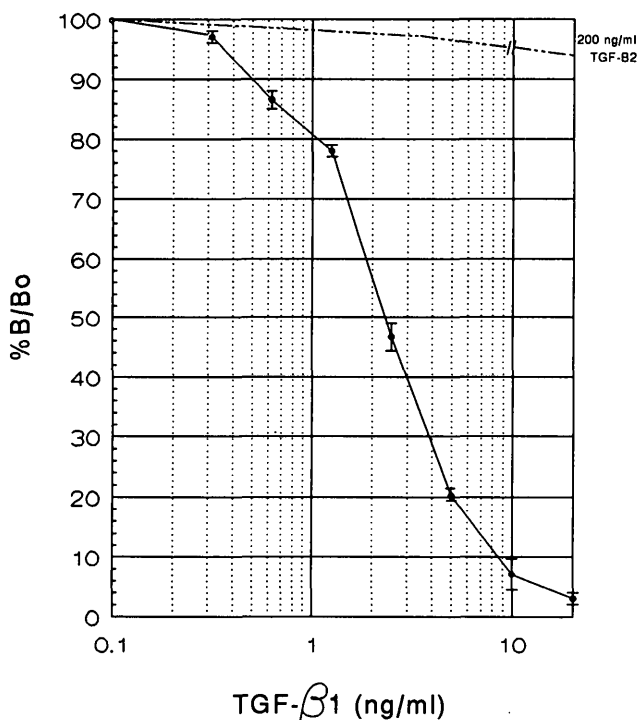
On the ethidium bromide-stained agarose gel, we obtained an extra band of TGF- $\beta$ 1 at the 400-base-pair position in cultured porcine trabecular cells. However, Southern hybridization with the oligonucleotide probe failed to detect this band. This additional product was not present in the sequences amplified from cDNA derived from whole trabecular meshwork, iris, or ciliary body, and it was too small to represent amplified fragments from contaminating genomic DNA, which would be expected to be about 718 base pairs.<sup>21</sup> Possible explanations include mispriming of PCR and an alternatively spliced product of the TGF- $\beta$ 1 mRNA that lost the specific probing sequence. In porcine tis-



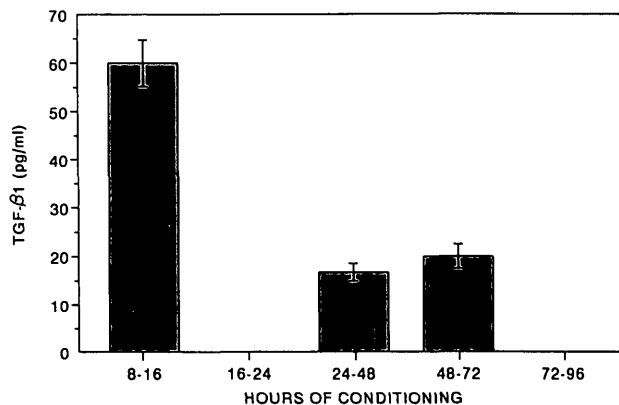
**FIGURE 4.** Southern hybridization of PCR-amplified products (from Fig. 2) with TGF- $\beta$ 1 antisense oligonucleotide probe specific for the sequence within the amplified segment. *Lane A* is the product from positive control DNA template, *lane B* from trabecular cells, and *lane C* from negative control. The length in base pairs corresponding to the DNA standard size marker is indicated on the left.

sues such as peripheral blood lymphocytes, the existence of more than one species of TGF- $\beta$ 1 mRNA has been reported;<sup>20</sup> these species are generated in a tissue-specific manner either by alternate splicing or by selection of heterogeneous 5' leader sequences. Cloning and nucleic acid sequencing are required for the conclusive characterization of the transcriptional regulation of TGF- $\beta$ 1 RNA in porcine trabecular cells.

By radioimmunoassay, we showed that trabecular cells in vitro synthesize and secrete TGF- $\beta$ 1. It is unlikely that the TGF- $\beta$ 1 we detected was a carry-over from serum, for several reasons. The cultures were initially washed twice with phosphate-buffered saline, and three times with serum-free medium. Furthermore, by the end of the third 8-hr incubation, no TGF- $\beta$ 1 was detected in the conditioned medium. The increased level thereafter clearly indicates the new secretion of TGF- $\beta$ 1 by the cultured cells into the supernatant. Thus, the mRNA for TGF- $\beta$ 1 detected in trabecular cells correlates with the biosynthesis of this cytokine. The TGF- $\beta$ 1 detected in the culture medium was present in a latent form, which implies that it is associated with binding proteins that are also produced by trabecular cells. Whether the presence of various substances, such as tissue plasminogen activators, cell surface-bound sialidases, and local acidic mi-



**FIGURE 5.** Radioimmunoassay calibration curve for TGF- $\beta$ 1 generated by use of known amounts of TGF- $\beta$ 1 standard and plotting of the means of duplicate determinations of the percentage bound (%B/Bo) as a function of the concentration of unlabeled TGF- $\beta$ 1. The lowest detectable level is 0.3 ng/ml, and cross-reactivity with TGF- $\beta$ 2 is < 6% at concentrations exceeding 200 ng/ml.



**FIGURE 6.** Concentrations of latent TGF- $\beta$ 1 in trabecular cell-conditioned medium at various times, as determined by radioimmunoassay and with the standard TGF- $\beta$ 1 calibration curve in Figure 5. The result obtained after 8–16 hr probably represents carry-over of TGF- $\beta$ 1 from serum-supplemented medium. TGF- $\beta$ 1 is produced and secreted in detectable amounts after the second 24-hr incubation period. No TGF- $\beta$ 1 was detected in nonactivated, conditioned medium.

croenvironments, in the trabecular meshwork is associated with the activation and usage of TGF- $\beta$ 1 in vivo requires further investigation. Our current results, together with those of previous studies,<sup>9-11</sup> suggest that the tissues bordering the anterior chamber may contribute to the level of TGF- $\beta$ 1 in the aqueous humor in health and disease.

The presence of TGF- $\beta$  in the anterior chamber of the eye has been considered to be primarily responsible for the induction of anterior chamber-associated immune deviation that renders this area an immunologically privileged site in which allogeneic tissue implants survive longer than they do in other sites of the body.<sup>5,6,23</sup> The synthesis of TGF- $\beta$  by cells of the iris and ciliary body is believed to generate anterior chamber-associated immune deviation.<sup>9,23</sup> The results of our study now implicate the trabecular cells as having a role in creating this unique intraocular microenvironment that influences the character of the immune responses to antigens in the eye.

Recently, we identified and quantified the high-affinity receptor for TGF- $\beta$ 1 on the trabecular cell,<sup>12,13</sup> and we demonstrated that TGF- $\beta$ 1 can have a potent inhibitory effect on the proliferation of trabecular cells.<sup>12</sup> These data suggest that TGF- $\beta$ 1 might have an autocrine and/or paracrine action within the trabecular meshwork. Although the exact actions of TGF- $\beta$ 1, and the mechanism for its activation in vivo remain to be elucidated, it is possible that, among other factors, the TGF- $\beta$  are significant in limiting the proliferation of trabecular cells of iris and ciliary body, as well as corneal endothelium in vivo.

TGF- $\beta$ 1 controls the accumulation of extracellular matrix macromolecules.<sup>1</sup> It activates gene tran-



scription and increases the synthesis and secretion of matrix proteins and protease inhibitors, while it decreases the synthesis of proteolytic enzymes that degrade matrix proteins. The effects on increased synthesis of matrix proteins are specific for TGF- $\beta$ , and both in vitro and in vivo experiments have demonstrated that it enhances the accumulation of types I, III, IV, V, and VII collagens,<sup>24-27</sup> fibronectin,<sup>25,28</sup> thrombospondin,<sup>29</sup> elastin,<sup>30</sup> and chondroitin/dermatan sulfate proteoglycans.<sup>31</sup> It is conceivable that this cytokine, once activated, can bind to its receptors on the trabecular cells and exert effects that lead to the excessive buildup of extracellular matrix proteins and to the decreased cellularity in the meshwork that occur with age and with the progression of primary open-angle glaucoma.<sup>32-34</sup> Therefore, quantitative PCR analysis of TGF- $\beta$ 1 mRNA in trabecular meshwork from normal aging eyes and from eyes with primary open-angle glaucoma would be beneficial for determination of any difference in the mRNA transcripts of this important growth modulator.

Previous investigations have shown that the trabecular meshwork, corneal endothelium, as well as most parts of the iris and ciliary body tissue are derived from neural crest.<sup>35-36</sup> Because TGF- $\beta$ 1 is the main growth factor that guides the migration of neural-crest cells to their final destination in the eye,<sup>37</sup> the presence of mRNA for TGF- $\beta$ 1 in trabecular cells is consistent with their embryonic origin. TGF- $\beta$  is also implicated directly in the modulation of human leukocyte antigen expression by certain types of cells.<sup>38-39</sup> The abnormal action of this cytokine on tissues of the eye could stimulate aberrant expression of human leukocyte antigens and consequently play a role in the pathogenesis of developmental disorders of the eye, particularly congenital glaucoma.<sup>37</sup>

Because of the multifunctional activities of TGF- $\beta$  and its possible involvement in the physiologic and pathologic processes of the eye, a great potential exists for the therapeutic application of this growth modulator, or of inhibitors, that suppress its inappropriate expression. The physiologic mechanisms for activation of the latent TGF- $\beta$ 1 and for regulation of expression of its mRNA as well as its receptors should be elucidated so the actions of this cytokine in health and disease can be understood.

### Key Words

trabecular meshwork, cell culture, polymerase chain reaction, Southern hybridization, radioimmunoassay, primary open-angle glaucoma

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