Inhibitory Effect of TGF-β2 in Human Aqueous Humor on Bovine Lens Epithelial Cell Proliferation

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Purpose. To determine whether transforming growth factor-β2 (TGF-β2) in human aqueous humor inhibits the proliferation of lens epithelial cells.

Methods. Bovine lens epithelial cells (BLECs) obtained from the central zone of the anterior capsule were cultured in complete medium (F-12 nutrient mixture supplemented with 5% fetal bovine serum). Human aqueous humor was obtained from 11 patients during cataract surgery. Medium was removed and replaced with medium containing various concentrations of porcine platelet-derived TGF-β2 or 10% human aqueous humor, with or without anti-TGF-β2 neutralizing antibody. The number of cells was counted at 96 hours.

Results. Porcine platelet-derived TGF-β2 inhibited BLEC proliferation in a dose-dependent manner. Anti-TGF-β2 antibody blocked the inhibitory effect of TGF-β2 on BLEC proliferation. The 10% human aqueous humor stimulated the proliferation of BLECs. Addition of anti-TGF-β2 antibody to the 10% human aqueous humor significantly enhanced this stimulatory effect on BLEC proliferation.

They had no other eye diseases except age-related cataract. The tenets of the Declaration of Helsinki were followed. Informed consent was obtained from all patients. There were seven men and four women, who ranged in age from 52 to 79 years (average age, 68.7 years). They had no other eye diseases except age-related cataract, and no systemic diseases. Specimens of aqueous humor were aspirated at the limbus with a 27-gauge needle during cataract surgery. In these patients, more than 0.2 ml of the aqueous humor could be aspirated. Specimens were immediately frozen at −70°C until used. Before cataract surgery, the patients were administered the following topical medications: dicrofenac sodium, tropicamide, and phenylephrine hydrochloride three times on the day of surgery, and antibiotics (norfloxacin) 3 times a day for 2 days.

To examine the effect of pure TGF-β2 on BLEC proliferation, we plated BLEC s (7000 cells/well) in 24-well plates (Falcon) and cultured them in complete medium. Twenty-four hours later, the cells of five wells were harvested after detachment with trypsin-EDTA (Gibco). The zero time cell count was determined with a Coulter Counter (Coulter Electronics, Luton, UK). Medium in the other wells was removed and replaced with 1.0 ml of the complete medium alone, or with medium containing 0.001, 0.0032, 0.01, 0.032, 0.1, 0.32, 1, 3.2, or 10 ng/ml TGF-β2. The number of cells was counted at 24-hour intervals for 4 days. The pure TGF-β2 was derived from porcine platelets and obtained from R&D Systems (Minneapolis, MN).

To determine whether anti-TGF-β2 neutralizing antibody blocked the effect of pure TGF-β2 and whether anti-TGF-β2 neutralizing antibody affected BLEC proliferation, the following experiment was performed using procedures similar to those described above. We plated BLEC s (7000 cells/well) in the 24-well plates. Twenty-four hours after plating, medium was removed and replaced with 1.0 ml of the complete medium containing anti-TGF-β2 neutralizing antibody (10 *g/ml), norfloxacin (10 *g/ml), a combination of TGF-β2 (1 ng/ml) and anti-TGF-β2 neutralizing antibody, or a combination of TGF-β2 and normal rabbit IgG. The combinations were incubated at room temperature 1 hour before the change of medium. Ninety-six hours later, cells were counted. Specimens of human aqueous humor were not pooled. We examined each specimen. A single determination of the paired sample (10% aqueous humor with anti-TGF-β2 neutralizing antibody or normal rabbit IgG) was made for each specimen because of the limited amount of aqueous humor. The effect of anti-TGF-β2 neutralizing antibody on each specimen of aqueous humor was expressed as the ratio of the number of cells cultured with a combination of 10% aqueous humor and normal rabbit IgG to that with a combination of 10% aqueous humor and anti-TGF-β2 neutralizing antibody.

Data are presented as mean ± SD. The Student’s paired t-test was used to analyze the difference of the effect of 10% aqueous humor. The two-tailed t-test with Welch’s correction was used for statistical analysis of other data. A level of P < 0.05 was accepted as statistically significant.

RESULTS

BLEC s proliferated in the complete medium. The zero time cell count of BLEC was 6700 ± 400 cells/well. Ninety-six hours after changing the medium, the number of cells cultured in the complete medium alone (control) increased to 24,000 ± 800 cells/well. This proliferation of BLEC was inhibited by pure TGF-β2 in a dose-dependent manner (Fig. 1). Having demonstrated the inhibition of pure TGF-β2 on lens epithelial cell proliferation, we next wanted to test whether TGF-β2 in human aqueous humor decreased the proliferation of lens epithelial cells.

Anti-TGF-β2 antibody (10 *g/ml) blocked the inhibitory effect of pure TGF-β2 (1 ng/ml) on BLEC proliferation. Anti-TGF-β2 (10 *g/ml) antibody alone had no effect on BLEC proliferation (Fig. 2).

The proliferation of BLEC was increased (1.13-fold) by adding 10% human aqueous humor and was significantly increased (1.35-fold) by adding a combination of 10% human aqueous humor and anti-TGF-β2 antibody. There was a statistically significant differ-
TGF-β2 antibody ranged from 1.06 to 1.35 (1.20 ± 0.08). Data are shown in Table 1.

DISCUSSION

Our study showed that pure TGF-β2 inhibits the proliferation of cultured lens epithelial cells in a dose-dependent manner (Fig. 1). The addition of anti-TGF-β2 antibody to 10% human aqueous humor significantly enhanced the stimulatory effect of the latter on BLEC proliferation (Fig. 3, Table 1). These results strongly support the presence of active TGF-β2 in human aqueous humor with the ability to decrease the proliferation of cultured lens epithelial cells.

Jampel et al. 3 detected TGF-β in human aqueous humor in amounts ranging from 2.3 to 8.1 ng/ml (4.5 ± 1.7 ng/ml), with 61% present in the active form and TGF-β2 as the main isoform. Cousins et al. 4 also reported the presence of 0.45 ng/ml active TGF-β in human aqueous humor, and they reported that most of its biologic activity was due to TGF-β2. Based on these data, the concentration of active TGF-β2 in 10% human aqueous humor would be expected to range from 0.05 to 0.5 ng/ml. Our results suggest that the amount of TGF-β2 in 10% human aqueous humor is sufficient to inhibit proliferation of lens epithelial cells. The dose of anti-TGF-β2 antibody that blocked 1 ng/ml TGF-2 was sufficient to block TGF-β2 in 10% human aqueous humor.

FIGURE 1. (A) Inhibition of bovine lens epithelial cell proliferation by pure TGF-β2. Values are mean ± SD of five wells. Bovine lens epithelial cells were cultured with complete medium alone (solid square) or medium containing 0.032 ng/ml TGF-β2 (solid circle), 0.1 ng/ml TGF-β2 (solid triangle), 0.32 ng/ml TGF-β2 (open circle), or 1 ng/ml TGF-β2 (open square). (B) Dose-dependent inhibition of bovine lens epithelial cell proliferation by pure TGF-β2. Cells were counted 96 hours after addition of TGF-β2. Values are mean ± SD of five wells.

FIGURE 2. Effects of anti-TGF-β2 antibody (AB, 10 μg/ml) and normal rabbit IgG (IgG, 10 μg/ml) on bovine lens epithelial cell proliferation cultured with complete medium (M) and M containing 1 ng/ml TGF-β2 (TGF). Values are means ± SD of five wells. *P < 0.001 compared with other three groups (two-tailed t-test).
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FIGURE 3. Effect of 10% human aqueous humor (AH) on proliferation of bovine lens epithelial cells. Cells cultured with complete medium (M) containing 10% PBS or AH with anti-TGF-β2 antibody (AB, 10 μg/ml) or normal rabbit IgG (IgG, 10 μg/ml). Values are means ± SD of 11 wells (11 wells in M + PBS + IgG, and 11 specimens in M + AH + IgG and M + AH + AB). *P < 0.001 versus M + PBS + IgG (two-tailed t-test). †P < 0.001 versus M + AH + IgG (Student’s t-test). ‡P < 0.01 versus M + PBS + IgG (two-tailed t-test).

It is unclear whether lens epithelial cells produce TGF-β2. If cultured BLECs produce TGF-β2, this TGF-β may modulate lens epithelial cell proliferation in our growth assay. However, TGF-β in the serum and that produced by cultured cells is in a latent form and has no effect until activated. In our experiment, 10 μg/ml anti-TGF-β2 antibody had no effect on BLEC proliferation. If active TGF-β2 was present in complete medium, it would be negligible in our growth assay.

TGF-β generally inhibits epithelial cell proliferation by lengthening or arresting the G1 phase of the cell cycle, and it does not appear to be cytotoxic. However, in some cell types, TGF-β decreases expression of mitogen receptors, such as EGF receptors in normal rat kidney rat fibroblast and platelet-derived growth factor receptors in bone marrow fibroblast. These effects may contribute to the growth-inhibitory response. In our growth assay, pure TGF-β2 inhibited the proliferation of lens epithelial cells, and anti-TGF-β2 neutralizing antibody promoted the mitogenicity of lens epithelial cells exposed to human aqueous humor. There is a possibility that some growth promoters or inhibitors other than TGF-β may cause part of this inhibition or promotion.

Another action of TGF-β is to upregulate cell adhesion by enhancing formation of extracellular matrix, which can modify the effect of TGF-β on cell proliferation indirectly. TGF-β stimulates rat fibroblast to grow in a semisolid medium by inducing the production of extracellular matrix, although this factor decreases the proliferation of these fibroblasts in monolayer culture. Richert et al reported that TGF-β indirectly stimulates cell division of chick lens annular pad cells by promoting cell spreading onto substrates.

Our finding that 10% human aqueous humor with anti-TGF-β2 antibody significantly stimulated the proliferation of lens epithelial cells indicates that human aqueous humor may contain one or more growth promoters (Fig. 3). Others have shown that growth factors such as bFGF, IGF, and EGF in aqueous humor promote the proliferation of lens epithelial cells in vitro. However, it is not known which factors may have sufficient stimulatory activity in human aqueous humor.

We did not examine the total dose of TGF-β (latent and active forms) because of the small quantity of specimens and because our goal was to determine whether human aqueous humor contained biologically active TGF-β2. Porcine, bovine, and murine aqueous humor contain low levels of active TGF-β, although they contain high levels of latent TGF-β. It is not known whether the latent TGF-β in aqueous humor may be activated in situ.

Ciliary epithelial cells produce TGF-β2 in vitro and in vivo. Lens fibers (but not lens epithelial cells) express TGF-β1 (but not TGF-β2) in the mouse embryo. The biologic function of these TGF-β isoforms in vivo is not yet known.

We have shown that pure TGF-β2 inhibits lens epithelial cell proliferation in vitro, and our indirect

TABLE 1. Effect of Anti-TGF-β2 Antibody on Proliferation of Bovine Lens Epithelial Cells Stimulated by 10% Human Aqueous Humor

<table>
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<tr>
<th>Patient Number</th>
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Human aqueous humor (AH), anti-TGF-β2 neutralizing antibody (AB; 10 μg/ml). Normal rabbit IgG (IgG; 10 μg/ml). Values represent a single determination.
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Key Words
TGF-β2, aqueous humor, lens epithelial cell, proliferation, inhibitory factor

References