Epidermal Growth Factor and Its Receptor, Basic Fibroblast Growth Factor, Transforming Growth Factor Beta-1, and Interleukin-1 Alpha Messenger RNA Production in Human Corneal Endothelial Cells

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The authors tried to determine whether human corneal endothelial cells in primary culture synthesize messenger RNA (mRNA) coding for epidermal growth factor (EGF), EGF receptor, basic fibroblast growth factor (FGFb), transforming growth factor beta-1 (TGFb1), and interleukin-1 alpha (IL-1 alpha). Oligodeoxythymidine-primed complementary DNA (cDNA) was generated from total cellular RNA extracted from eight independent primary corneal endothelial cell cultures. Four of these cultures, maintained 18–51 days, had obvious increases in cell numbers and mass over the 2 weeks before RNA extraction and were populated primarily with cells that were small, uniform, and mononuclear (proliferative cultures). The morphology of the cells in other four cultures, maintained 47–78 days, was predominantly large, irregular, vacuolated, and occasionally multinucleated. These cells were identical to senescent cells found in previous studies, and the cell number did not increase in these cultures over the 2 weeks preceding RNA extraction (senescent cultures). The polymerase chain reaction (PCR) was used to amplify the growth factors (EGF, FGFb, TGFb1, and IL-1 alpha), EGF receptor, and beta actin sequences from each of the cDNA samples. The EGF receptor, FGFb, and beta actin mRNAs were present in all eight cDNA samples. The EGF mRNAs were detected by PCR alone in four of the samples from proliferative cultures, TGFb1 mRNAs in three, and IL-1 alpha mRNAs in three. In the samples from senescent cultures, 0, 1, and 0 mRNAs were detected, respectively. Southern blots of the PCR products were probed with oligonucleotides complementary to sequences in each of the amplified products. This technique showed that the appropriately sized amplification products were specific. These results demonstrate that cultured human corneal endothelial cells produce mRNA for EGF, EGF receptor, FGFb, TGFb1, and IL-1 alpha. The EGF, IL-1 alpha, and TGFb1 mRNAs were diminished in senescent cells, suggesting that decreases in the endogenous production of these growth factors may contribute to the corneal endothelial cell senescence. Production of both EGF and EGF receptor mRNA suggested an autocrine role for EGF in corneal endothelial cell physiology. Invest Ophthalmol Vis Sci 32:2747–2756, 1991

Despite the critical importance of the endothelial cell layer to the maintenance of normal corneal hydration, many studies suggest that human endothelial cells do not divide at a sufficient rate to maintain a constant density. The number of human corneal endothelial cells appears, in general, to decrease with increasing age and after insults such as those from trauma and intraocular surgery. In young children, the endothelial cell density varies from 2000–4000 cells/mm². There tends to be a decrease in endothelial cell density with increasing age after childhood that varies in individual subjects. After wounding, human corneal endothelium appears to heal primarily by enlargement and migration. Although recent studies showed that human corneal endothelial cells are capable of limited in vivo mitosis, cell division is thought to contribute relatively little to healing. There is limited information available regarding factors that might regulate human corneal endothelial cells and endothelial cells from other species such as the rabbit that has a greater proliferative potential.
Contributing to a lack of knowledge regarding human corneal endothelial cell growth and senescence is the limited information that is available regarding the endogenous modulators produced by these cells. The available data, for the most part, were provided by three studies. One showed that bovine corneal endothelial cells contain poly(A)-positive RNA coding for bioactive basic fibroblast growth factor (FGFb) and suggested that FGFb produced by these cells may act in an autocrine or paracrine manner to stimulate proliferation. Similarly, another found that FGFb protein is produced by bovine corneal endothelial cells. Others used radiolabeled epidermal growth factor (EGF) to show that EGF receptors are present on the surface of feline and human endothelial cells. Growth factors and growth factor receptors have important roles in regulating cell growth in many types of cells. It is essential to have a more complete knowledge of the endogenous production of growth factors and growth factor receptors by human corneal endothelial cells to understand the limited capacity of these cells for proliferation.

A major problem impeding progress in this area was the limited availability of human tissue for experiments done with traditional molecular biologic and biochemical techniques. Our results obtained with the sensitive and specific polymerase chain reaction (PCR) technique show that human corneal endothelial cells in primary culture synthesize mRNAs coding for EGF, EGF receptor, FGFb, transforming growth factor beta-1 (TGFb1), and interleukin-1 alpha (IL-1 alpha). Furthermore, EGF, TGFb1, and IL-1 alpha coding mRNAs were found to vary in cultured human corneal endothelial cells with differing morphology. The mRNAs for these growth factors tend to be diminished in cultures populated primarily with human corneal endothelial cells that have primarily a large, irregular, vacuolated, and sometimes multinucleated morphology (characteristic of senescent cells) compared with cells with a small, uniformly shaped morphology characteristic of human corneal endothelial cells that are proliferating from explants.

**Materials and Methods**

Corneas stored for less than 96 hr in Optisol or Dexsol (Chiron Ophthalmics, Irvine, CA) were obtained from eyebanks. Those included were judged to be of transplant quality but were excluded from clinical use because of advanced donor age or other nonocular exclusion criteria. All corneas were from donors who were older than 40 years of age. The corneas were punched from the endothelial side with a 10-mm trephine, and the button was bisected with a no. 22 scalpel blade. The Descemet's membrane-endothelial complex was stripped from the stroma of each half button using fine forceps, rinsed twice with Hank's balanced salt solution, and cut into approximately ten strips with a scalpel blade. All strips from one cornea were transferred to a Falcon Primaria T-25 flask (Becton Dickinson Labware, Lincoln Park, New Jersey) containing 1.5 ml of media. Endothelial cells were cultured, according to a method previously described, in culture medium containing 10% fetal bovine serum and 5% calf serum. All cell culture reagents were obtained from Hazelton Biologics (Le-

**Fig. 1.** Inverted phase-contrast micrograph of human corneal endothelial cells with the small, uniform morphology (culture 2, 25 days) in primary culture. Original magnification x450.
nexa, KS). The cells were fed twice a week with complete medium.

Total cellular RNA was isolated from each of eight primary cultures with approximately 20–40% coverage of the T-25 flask by endothelial cells. Four of the cultures (cultures 1–4) were maintained for 18, 25, 43, and 51 days, respectively; they had obvious increases in cell numbers over the 2 weeks before RNA extraction and were populated primarily with corneal endothelial cells with a small, uniform morphology (Fig. 1). These cultures were referred to as proliferative cultures. Four cultures (cultures 5–8) were maintained for 47, 58, 65, and 78 days; they contained a high proportion of corneal endothelial cells with a large, irregular, vacuolated, and sometimes multinucleated morphology (Fig. 2). The latter cultures were observed to have minimal increases in cell numbers over the 2 weeks before RNA extraction. These cultures were referred to as senescent cultures. During RNA extraction, the cells were rinsed twice with Hank’s balanced salt solution, and 1.5 ml of guanidinium thiocyanate (GTC) solution was transferred onto the

Fig. 2. (A) Inverted phase-contrast micrograph of human corneal endothelial cells with the large, irregular, vacuolated, and sometimes multinucleated morphology (culture 6, 58 days) in an area of the plate with relatively high cell density. (B) Micrograph of another area of culture 6 with lower cell density, where individual cells with the large, irregular, vacuolated, and sometimes multinucleated morphology are better appreciated. Original magnifications ×450.
monolayer. The GTC cellular extract was passed 15 times through a 22-gauge needle and layered over 0.6 ml of 6.2 M cesium chloride solution in an 11 × 34-mm polyallomer centrifuge tube (Beckman, Palo Alto, CA). The sample was centrifuged at 55,000 rpm (2 × 10^5 × g) in a TLS-55 swinging bucket rotor (Beckman) for 6 hr at 22°C in a TL-100 ultracentrifuge (Beckman). Otherwise the extraction was as previously published. After ethanol precipitation, the RNA was resuspended in 20 µl of water. All solutions used for the isolation or subsequent manipulation of RNA were prepared in water purified with the Milli-Q UF+ system (Millipore, Bedford, MA), treated with a 0.001 volume of diethylylpyrocarbonate at 37°C for 12 hr, and autoclaved to inhibit RNase activity. Tris HCl-containing solutions were prepared in similarly treated distilled water.

First-strand complementary DNA (cDNA) was prepared from total cellular RNA using a previously published method. For each sample, the reverse transcriptase reaction was done in a single 100-µl reaction including all of the RNA isolated from an individual culture flask, 1200 ng of oligododeoxythymidine, primer (Promega, Madison, WI), 50 mM Tris HCl, 75 mM potassium chloride, 10 mM dithiothreitol, 3 mM magnesium chloride, deoxyribonucleotides (deoxyadenosine triphosphate, deoxythymidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate, each at 0.625 mM; Promega), 40 units of avian myeloblastosis virus (AMV) reverse transcriptase (Promega), and 80 units of RNAsin (Promega).

The PCR primers for IL-1 alpha and beta actin were synthesized (Midland Certified Reagent, Midland, TX) using oligonucleotide sequences described in previous studies (Table 1). The PCR primers for EGF, FGFb, TGFb1, and EGF receptor were designed so that TGF alpha sequences, with which EGF has considerable sequence homology, would not be detected. All six primer pairs were designed so that amplification of contaminating genomic DNA sequences would produce PCR products that were substantially larger than PCR products amplified from cDNA; intron sequences that were excised during RNA processing would be included in genomic DNA targets. For beta actin, for example, amplification of the genomic sequence would have yielded a PCR product that was 790 base pairs in length compared with 350 base pairs for the cDNA amplification product (Table 1). Each upstream and downstream primer was synthesized beginning at the 5' end with a cytosine-thymine-cytosine (CTC) clamp, followed by a restriction enzyme recognition site that was absent from the amplified sequence (to allow for future cloning of the PCR products), and the priming sequence (Table 1). The CTC clamp was included to facilitate the future cutting of the PCR amplification products with restriction enzymes because the terminal few nucleotide pairs of a DNA molecule may have broken hydrogen bonds between paired nucleotides on opposite strands some of the time. We did PCR amplification of each sequence on 5 µl of endothelial cDNA sample in a total volume of 100 µl using 2 units of Tag polymerase (Perkin-Elmer Cetus, Norwalk, CT) and 1.5 mM magnesium as previously described. Control reactions without template were included in PCR amplifications with each primer set. Programmable temperature cycling (Ericomp, La Jolla, CA) was done with the following cycle profile: denaturation 4 min at 94°C, followed by 40 cycles of annealing 2 min at 55°C, extension 3 min at 72°C, and denaturation 90 sec at 94°C. Unless otherwise specified, all reagents were obtained from Sigma (St. Louis, MO). The PCR reactions that yielded no product were repeated with parallel positive control reactions for confirmation.

Horizontal 1.5% agarose (US Biochemical, Cleveland, OH) gel electrophoresis was done by a previously described technique using 18 µl of each PCR product and 2 µl of tenfold-concentrated loading buffer per lane with a 70-µl gel run in a wide Mini-

### Table 1. Polymerase chain reaction primers and hybridization probes

<table>
<thead>
<tr>
<th>Modulator</th>
<th>Ref</th>
<th>UP primer</th>
<th>DP primer</th>
<th>Probe</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>22</td>
<td>3119–3139(NotI)</td>
<td>3491–3511(NotI)</td>
<td>3377–3406</td>
<td>415</td>
</tr>
<tr>
<td>EGF</td>
<td>25</td>
<td>2891–2910(HindIII)</td>
<td>4010–4029(Kpnl)</td>
<td>3184–3213</td>
<td>1157</td>
</tr>
<tr>
<td>FGFb</td>
<td>23</td>
<td>415–434(EcoRI)</td>
<td>799–818(HindIII)</td>
<td>565–594</td>
<td>422</td>
</tr>
<tr>
<td>TGFb1</td>
<td>24</td>
<td>1749–1769(NotI)</td>
<td>1972–1992(NotI)</td>
<td>1765–1794</td>
<td>266</td>
</tr>
<tr>
<td>IL-1a</td>
<td>20</td>
<td>10–33(BamHI)</td>
<td>697–720(PstI)</td>
<td>502–531</td>
<td>729</td>
</tr>
<tr>
<td>Beta actin</td>
<td>21</td>
<td>1628–1650(EcoRI)</td>
<td>2433–2400(Xhol)</td>
<td></td>
<td></td>
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UP and DP primers denote the nucleotide numbers used in synthesizing the upstream and downstream primers in the specified reference. Restriction enzyme in parentheses represents the site included after the clamp site at the 5' end. Probe denotes the nucleotide numbers for the oligonucleotide probes used in confirming the identity of the amplified PCR fragment using Southern blotting. Size indicates the expected size of the amplified sequence including the 5' clamps and restriction sites. The size of the beta actin fragment does not correspond to the nucleotide numbers, since the referenced sequence is for genomic DNA and includes an intron that is excised during RNA process.
Subcell electrophoresis apparatus (Bio Rad, Richmond, CA). Phi x 174 RF DNA/Hae III fragments (Bethesda Research Laboratories, Gaithersburg, MD) were used as molecular size standards. Ten μl of 10 mg/ml ethidium bromide were added to the running buffer, and a voltage of 80 V was applied until the loading dye had traveled two thirds of the distance to the end to the gel (approximately 90 min).

Southern hybridization was done to confirm that the PCR amplified sequences were derived from RNA coding for EGF, EGF receptor, FGFb, TGFb1, and IL-1 alpha using 30-base oligonucleotide probes that hybridized to regions in the amplified sequences (Table 1). We applied 5 μl of PCR product to each lane and resolved it with 1.5% agarose gel electrophoresis.29 Phi x 174 RF DNA/Hae III fragments were used as molecular size standards. Separated PCR amplification products were deposited on a supported 0.45-μm nitrocellulose filter (Nitroplus 2000; MSI, Westboro, MA) by capillary transfer with 20-fold concentrated SSC, single-concentrated Denhardt’s solution (sixfold concentrated SSC, single-concentrated 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0).29 The DNA was fixed to the filter by baking at 80°C for 2 hr. Oligonucleotide 5’ radiolabeling and hybridization were done by a previously published method.30 31 Briefly, 165 ng of oligonucleotide probe were labeled at the 5’ end using gamma-32P adenosine 5’-triphosphate (>7000 Ci/mmol; ICN, Costa Mesa, CA) and bacteriophage T4 polynucleotide kinase (Promega). The Southern blot was prehybridized for 2 hr at 37°C with 0.5 ml/cm2 prehybridization solution (sixfold concentrated SSC, single-concentrated Denhardt’s solution, 0.5% sodium dodecyl sulfate, 0.05% sodium pyrophosphate, and 100 μg/ml sheared salmon sperm DNA). Hybridization was done with the labeled oligonucleotide (106-107 cpm/ml) in 0.2 ml/cm2 hybridization solution (sixfold concentrated SSC, single-concentrated Denhardt’s solution, 0.05% sodium pyrophosphate, and 20 μg/ml sheared salmon sperm DNA) at 68°C for 5 hr. After hybridization the blot was washed with sixfold concentrated SSC and 0.05% sodium pyrophosphate for 30 min at 37°C, 30 min at 50°C, and 20 min at 68°C. Autoradiography was done at −70°C with Kodak (New Haven, CT) OMAT-AR film with an intensifying screen.

### Results

Nearly 50% of the primary endothelial cell cultures (16 of 33) had outgrowth to cover approximately 20–40% of the culture flask in 2–12 weeks. Previous studies reported similar rates of success in corneas from older individuals.17,32 In these primary cultures, initial growth was characterized by small, uniformly shaped cells proliferating around explants (Fig. 1). This morphology was characteristic of cultures in which there was an obvious increase in cell number and mass with time. After an additional period of time varying from approximately 2–8 weeks, proliferation decreased markedly in all cultures. At this point, the cultures were characterized by a high proportion of cells that were large, irregular, vacuolated, and sometimes multinucleated (Fig. 2). These cells were similar to senescent corneal endothelial cells seen in previous studies.17,32

Six primary human corneal endothelial cell cultures that had senescent morphology were maintained with media changes twice a week for several months. In each case, obvious decreases in cell mass occurred in the culture over time after the predominant morphology became the senescent type. Two of these cultures had no cells remaining after 4 months.

Figure 3 shows the PCR products generated from a cDNA sample produced from a primary human cor-

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Fig. 3. PCR products amplified from the cDNA samples generated from a human corneal endothelial primary culture (culture 2) with the small, uniform, proliferative morphology and a culture (culture 6) with the large, irregular, senescent morphology. Lanes with PhiX174/HAEIII size markers are indicated αX. The lengths of appropriate markers in base pairs are provided to the left. The primers used to generate the PCR products (Table 1) are indicated above each lane. Each of the appropriately sized products was detected in the proliferative culture. Only EGF receptor, FGFb, and beta actin were detected by PCR in this culture with senescent morphology. The arrow designates the expected 266 base pair band for TGFb1. The smaller TGFb1-primed band present at approximately 210 base pairs was not detected by Southern blotting (not shown).
neal endothelial cell culture (culture 2, lanes 2–7) populated predominantly with cells having the small, uniform morphology (Fig. 1) and a cDNA sample from a culture (culture 6, lanes 8–13) populated with human corneal endothelial cells having predominantly the large, irregular, vacuolated, and sometimes multinucleated morphology (Fig. 2). Figure 3 demonstrates that amplification products of the expected length were detected for each of the growth factor and growth factor receptor sequences. Figure 4 shows the PCR products amplified for each growth factor and growth factor receptor from the cDNA prepared from the four cultures with the proliferative morphology (cultures 1–4) and the four with the senescent morphology (cultures 5–8). The PCR products of the expected sizes were amplified for EGF receptor, FGF, and beta actin from cDNA produced from all of the primary cultures, including four with the proliferative cell morphology and four with the senescent morphology. The EGF mRNAs were detected by PCR in four of the proliferative samples, TGFβ1 mRNAs in three, and IL-1 alpha mRNAs in three. In the senescent samples, 0, 1, and 0 mRNAs were detected, respectively.

Hybridization of Southern blots of the PCR products with oligonucleotide probes complementary to 30-nucleotide intervals in the amplified EGF, EGF receptor, FGF, TGFβ1, and IL-1 alpha sequences (Table 1) detected molecules of the expected sizes (Fig. 5) and provided confirmation that the amplified sequences were specific. For IL-1 alpha, an additional band was detected on the Southern blot at 968 base pairs in size was also detected. This latter band did not appear on the ethidium bromide stained gel (Fig. 3).

![Fig. 4. PCR products for each of the cDNA samples generated from human corneal endothelial primary cultures. Each column contains the PCR products generated from a single oligo-dt-primed cDNA sample. Columns 1–4 are from cultures (1–4, respectively) populated primarily with endothelial cells with the small, uniform, proliferative morphology. Columns 5–8 are from cultures (5–8, respectively) populated primarily with endothelial cells with the large, irregular, and vacuolated morphology. Lane 9 is the control lane without target for each pair of PCR primers. Row designations are the PCR primers used to amplify the cDNA. Bands in column 1 for TGFβ1 and in column 4 for FGF appear faint, but were easily observed on the original ethidium bromide-stained agarose gel. Negative PCR reactions were repeated along with positive controls for confirmation.](https://lov.s.arvojournals.org)

![Fig. 5. Southern blots of the PCR products from Figure 4. Blots of PCR products for each growth factor or growth factor receptor amplification were probed with oligonucleotides specific for a sequence within each amplified segment (Table 1). Columns 4 and 3 are products from cultures with proliferative morphology (4 and 3, respectively, except cultures 2 and 4, from left to right, were included for TGFβ1 [arrowheads]). Columns 7 and 6 are products from senescent cultures (7 and 6, respectively, except the order was 6 and 7 for TGFβ1 [asterisks]). Row designations indicate PCR products and probe used in Southern hybridization. Each labeled PCR product was of the expected length, based on the design of the PCR primers (Table 1). The arrow denotes the 729 base pair band for IL-1a. A larger amplification product approximately 968 base pairs in size was also detected. This latter band did not appear on the ethidium bromide stained gel (Fig. 3).](https://lov.s.arvojournals.org)
blotting of the PCR products from proliferative culture 4 (Fig. 5), although they were not easily detected on the agarose gel (Fig. 4, column 4, rows TGFβ and IL-1 alpha). In Figure 5, however, the mRNA sequences of the expected size coding for TGFβ1 and IL-1 alpha were not detected in two samples with senescent morphology even with Southern blotting of the PCR products. These results underscore the low levels of EGF, TGFβ1, and IL-1 alpha mRNAs in cultures with a high proportion of cells with senescent morphology.

Discussion

Our results showed that human corneal endothelial cells synthesize mRNA coding for EGF receptor. This was consistent with the findings of a previous study,16 showing that EGF receptor protein is present in human corneal endothelium. This study also found that FGFβ mRNA, previously shown to be produced by bovine endothelium,14 also is produced by cultured human corneal endothelial cells. We obtained conclusive evidence that mRNAs coding for EGF, TGFβ1, and IL-1 alpha are produced by cultured human corneal endothelial cells. Although identification of specific mRNAs in the endothelial cells suggests that there is synthesis of the corresponding proteins, unequivocal evidence of translation of the sequences to produce the bioactive proteins will require further investigation.

We also detected differences in the levels of the mRNAs coding for EGF, TGFβ1, and IL-1 alpha between cultures populated by cells with differing morphology. These mRNAs tended to be diminished in cultures populated predominantly by human corneal endothelial cells with a large, irregular, and vacuolated morphology characteristic of senescent cells (Fig. 2) compared with cultures populated predominantly by cells that were small, uniform in size, and increasing in number (Fig. 1). Even though the transcripts for EGF receptor, FGFβ, and beta actin consistently were identified in all cultures, EGF, TGFβ1, and IL-1 alpha mRNAs could not be detected in some cultures with the senescent morphology with the sensitive PCR method augmented by Southern blotting of the PCR products. We do not know whether high levels of TGFβ1 mRNA appeared to be present in one of the cultures with senescent morphology. Further work is needed to determine the roles of these endogenous modulators in human corneal endothelial cell growth, senescence, and morphology.

Cell counts were not done to avoid possible effects of trypsinization on gene expression. Also, absolute cell counts would have been of questionable significance for comparisons of cell mass between cells with proliferative and senescent morphology that differed dramatically in the sizes of individual cells (Figs. 1, 2). Although we could not determine the cell mass, each of the cultures had a total coverage of the culture flask that fell within a similar range. Beta actin was used as an internal control to provide some indication that the cell masses in the cultures also were comparable. Many studies show that the amount of the cytoskeletal protein actin is directly proportional to the cell size and that the rate of actin mRNA transcription is relatively constant when compared with other mRNAs.33-35 In the first study,32 relative rates of synthesis of actin did not differ between proliferating and confluent cultured bovine corneal endothelial cells. Figure 4 shows that, using PCR, beta actin was detected with similar efficiency in each culture. The detection of beta actin, along with EGF receptor and FGFβ, in all of the senescent cultures suggests that mRNAs for EGF, TGFβ1, and IL-1 alpha also should have been detected in each of the senescent cultures if these mRNAs were present at levels that were similar to those found in cultures with proliferative morphology.

Amplification products of two different sizes were detected during the evaluation of IL-1 alpha PCR reactions by Southern blotting using the IL-1 alpha-specific oligonucleotide probe. The smaller 729 base pair band was the expected reaction product for IL-1 alpha. The identity of the larger 968 base pair reaction product is unknown. Its presence, however, does not complicate the conclusion that the expected IL-1 alpha mRNA was produced in corneal endothelial cells. The larger sequence was present in lower concentration in the PCR reaction products because it was not detected on the agarose gel (Fig. 3). It was amplified by the primers for IL-1 alpha and recognized by the IL-1 alpha-specific probe, therefore, it had a significant degree of sequence similarity to the expected IL-1 alpha product. It is possible that this is an alternative RNA splicing product for the primary IL-1 alpha transcript. Nucleic acid sequencing will be needed to demonstrate the relationship between these products conclusively.

Because the PCR methods used in these experiments27,28 were sensitive only to large changes in the levels of mRNA molecules, there could have been less pronounced, but significant, alterations in the levels of EGF receptor, FGFβ, and beta actin mRNA between proliferative and senescent endothelial cells. More precise measurements of the levels of each of these modulators can be made in human corneal endothelial cells using control sequences that are being developed for quantitative PCR.36 Although quantitative PCR is technically more complex, the application of these methods should provide additional insights into the relationship between endogenous
growth factor and growth factor receptor production and human endothelial cell senescence.

The functions of EGF, EGF receptor, FGFb, TGFb1, and IL-1 alpha in the corneal endothelial cells are uncertain. Many studies reported proliferative responses in human and animal corneal endothelial cells in response to exogenous EGF and FGFb and TGFb1. The continued expression of EGF receptor mRNA by cells that have the senescent morphology suggests that these cells might respond to exogenous EGF. The production of both EGF and EGF receptor mRNAs in cells with proliferative morphology suggests an autocrine role for EGF in human corneal endothelial cells in culture. In addition, FGFb, TGFb1, and IL-1 alpha may have autocrine effects on corneal endothelial cell functions. Further work is needed to determine whether receptors for FGFb, IL-1 alpha, and TGFb1 (the molecular sequence for TGFb1 receptor has not been found) are expressed by corneal endothelial cells. Protein precursors for FGFb and IL-1 alpha, however, lack classic signal peptide sequences characteristic of other extracellular modulators. The recent detection of FGFb and IL-1 alpha proteins in the nuclei of other cell types, therefore, suggested that, in some cells, these factors may function as intracellular modulators of gene expression that are not mediated through cell-surface receptors.

The effects of growth factors on cellular functions commonly differ depending on the cell type. For example, IL-1 alpha inhibits the proliferation of vascular endothelial cells, but it stimulates fibroblasts. In a recent study of vascular endothelial cells, IL-1 alpha was present in high levels in senescent cells and had a role in maintaining the senescence of these cells through its inhibitory effects on proliferation. We detected higher levels of the IL-1 alpha mRNA molecule in corneal endothelial cells with a proliferative rather than senescent morphology. This does not necessarily suggest that there is a stimulating effect of IL-1 alpha on human corneal endothelial cells. The IL-1 alpha could inhibit proliferation and prevent a more exuberant response than is generally seen with human corneal endothelial cells in culture. Similarly, nothing is known regarding the effects of TGFb1 on corneal endothelial cell proliferation. More investigation is needed to define the roles of IL-1 alpha and TGFb1 on the growth of human corneal endothelial cells.

Although relatively little is known regarding corneal endothelial cell senescence, progress has been made in understanding senescence in other types of cultured cells. Cell-fusion experiments (in which senescent cells were fused to either actively growing cells of the same type or to a different immortal cell line) showed that the senescent phenotype is dominant and suggested that an active factor(s) inhibiting proliferation is present in the cytoplasm of the senescent cell. It also was found that protein coding poly(A)-positive RNA from senescent cells inhibits mitosis when microinjected into rapidly dividing cells. Recently, senescent vascular endothelial cells that did not respond to exogenous growth factors were shown to produce IL-1 alpha mRNA; young vascular endothelial cells did not produce the IL-1 alpha transcript. When an antisense oligonucleotide complementary to the mRNA for IL-1 alpha was included in the growth media of presenescent cells, senescence was inhibited, and there was extension of the proliferative life span. The antisense oligonucleotide hybridized to the IL-1 alpha mRNA and prevented synthesis of IL-1 alpha protein. These studies suggest that senescence in vascular endothelial cells is an active, genetically programmed process that can be manipulated and that the expression of growth-modulating proteins controlling cell division may play a fundamental role in the process. Large variations in the levels of mRNA transcripts for EGF, TGFb1, and IL-1 alpha imply that similar mechanisms may have a role in the development of senescence in corneal endothelial cells.

Key words: corneal endothelium, epidermal growth factor, epidermal growth factor receptor, basic fibroblast growth factor, transforming growth factor beta-1, interleukin-1 alpha, polymerase chain reaction

References


