Fibroblast Growth Factor Receptor-1, Interleukin-1 Receptor, and Glucocorticoid Receptor Messenger RNA Production in the Human Lacrimal Gland

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**Purpose.** To determine whether messenger RNA coding for fibroblast growth factor receptor-1, interleukin-1 receptor, and glucocorticoid receptor messenger RNA are produced in human lacrimal tissue.

**Methods.** Total cellular RNA was isolated from three specimens of normal human lacrimal tissue and complementary DNA was synthesized. The polymerase chain reaction and sequence-specific primers were used to amplify the sequences of interest from the complementary DNA. Hot blotting and sequence-specific probes were used to demonstrate that the expected amplification products were specific.

**Results.** Data demonstrated that fibroblast growth factor receptor-1, interleukin-1 receptor, and glucocorticoid receptor messenger RNA are produced in human lacrimal tissue.

**Conclusions.** These results and the previous identification of basic fibroblast growth factor in the lacrimal gland suggest that basic fibroblast growth factor has autocrine or paracrine functions in lacrimal tissue. More study is needed to determine whether the corresponding proteins are produced and, if so, what functions are regulated by fibroblast growth factor receptor-1, interleukin-1 receptor, and glucocorticoid receptor in the lacrimal gland.

Recent studies have demonstrated that epidermal growth factor (EGF) messenger RNA (mRNA) and protein are produced in human lacrimal gland tissue. In addition to possible exocrine effects of EGF produced by the lacrimal gland on the ocular surface, the identification of EGF receptor mRNA suggested that EGF may have autocrine or paracrine effects in the lacrimal gland. Lacrimal tissue was also found to produce mRNA coding for basic fibroblast growth factor (FGF). We have used the polymerase chain reaction and hot blotting methods to demonstrate that human lacrimal tissue also produces mRNA coding for FGF receptor-1, the interleukin-1 (IL-1) receptor, and the glucocorticoid receptor.

**METHODS**

Normal human lacrimal tissue was obtained from three patients who had orbital lobe lacrimal gland biopsies performed at the time of blepharoplasty. The research followed the tenets of the Declaration of Helsinki, informed consent was obtained from each pa...
tient before surgery after the nature and the possible consequences of the study were explained, and the research project was approved by the Investigational Review Board at the University of Texas Southwestern Medical Center. Surrounding connective tissue was carefully dissected from each specimen. The tissue was rinsed with balanced salt solution, transferred to a cryovial, and immediately frozen in liquid nitrogen.

Total cellular RNA was isolated from the frozen lacrimal tissue and primary human corneal epithelial cells using the guanidinium method. First-strand complementary DNA (cDNA) was synthesized with approximately 10 μg of lacrimal gland total cellular RNA in a 100 μl reaction using AMV reverse transcriptase (Promega Corporation, Madison, WI). Six hundred nanograms of oligo (dt)15 (Promega) was included as the cDNA primer. Solutions used for RNA isolation and cDNA synthesis were treated with 0.001 volume of diethylpyrocarbonate (Sigma, St. Louis, MO) at 37°C for 12 hr and placed in an autoclave to inhibit RNase activity. Tris-HCl-containing solutions were prepared in similarly treated distilled water.

Sequences of interest were amplified from human lacrimal gland and corneal epithelial cDNA using the polymerase chain reaction (PCR) in a 100 μl reaction volume with 1.5 mM magnesium chloride. Five μl of cDNA reaction product and two units of Taq polymerase (Perkin Elmer Cetus Corporation, Norwalk, CT) were included in each PCR reaction. Control reactions were performed without template for each PCR primer pair during each amplification. Programmable temperature cycling (Ericomp Corp., La Jolla, CA) was performed with the following cycle profile: initial 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.

PCR primers for FGF receptor-1 have been described previously (primers P1a and P1b in the cited work). The FGF receptor-1 primers distinguish between three amino-terminal motifs of the FGF receptor-1 proteins that are derived by alternative splicing of a single genomic sequence. The 1100 base-pair PCR amplification product corresponds to the alpha amino-terminal motif of FGF receptor-1 containing three extracellular immunoglobulin G (IgG)-like disulfide loops. The 800 base-pair product corresponds to the beta amino-terminal motif of FGF receptor-1 containing two extracellular IgG-like disulfide loops. Finally, the 1000 base-pair amplification product corresponds to the gamma amino-terminal motif of FGF receptor-1. This motif does not contain a known signal sequence for membrane translocation and is believed to represent an intracellular form of FGF receptor-1.

PCR primers for the interleukin-1 receptor were designed using the known messenger RNA sequence. The upstream primer was nucleotides 4284-4303 [TGA-CAA-AAT-TGC-CCA-GAG-AG] and the downstream primer was the inverse complement of nucleotides 4643-4662 [sequence of primer 5’ to 3’ TTG-TGC-TAA- ACC-AGT-TTT-AA]. A CTC clamp and restriction enzyme site (upstream, Xhol: downstream, BamHI) were included at the 5’ end of each primer to facilitate molecular cloning for future studies. The IL-1 receptor primers were designed so that amplification of the messenger RNA would produce a PCR product that was 397 base pairs in length. The genomic organization of the IL-1 receptor gene has not been reported.

PCR primers for the glucocorticoid receptor were designed using the known messenger RNA sequence. The upstream primer was nucleotides 1324-1343 [ATG-AGA-CCA-GAT-GTA-AGC-TC] and the downstream primer was the inverse complement of nucleotides 1980-1949 [sequence of primer 5’ to 3’ AAT-GGC-ATA-AGA-AC-ATC-CA]. A CTC clamp and restriction enzyme site (upstream, Xhol: downstream, BamHI) were included at the 5’ end of each primer. The glucocorticoid receptor primers were designed so that amplification of the messenger RNA would produce a PCR product that was 643 base pairs in length and amplification of the genomic sequence would yield PCR product that was much larger because it would include 3 intron sequences.

PCR primers for beta actin were synthesized using oligonucleotide sequences described in a previous study. These beta actin PCR primers were designed so that the corresponding genomic DNA sequences would include intron sequences. PCR amplification of any genomic DNA contaminating the total cellular RNA preparation would therefore yield sequences significantly larger than the amplified regions from cDNA. The genomic DNA amplification for beta actin would be 790 base pairs in length compared to the 350 base pairs for the cDNA amplification product.

Agarose (United States Biochemical Corp., Cleveland, OH) gel electrophoresis was performed using 27 μl of each PCR product and 3 μl of 10X loading buffer per lane with a 150 ml 1% gel run in a wide Mini-Sub cell electrophoresis apparatus (Bio Rad, Richmond, CA). One hundred base-pair–increment DNA fragments (Bethesda Research Laboratories, Gaithersburg, MD) were used as molecular size standards. Ethidium bromide 10 μl of 10 mg/ml (Bio Rad, Richmond, CA) was added to the running buffer and 100 volts applied until the leading marker dye had been transported approximately 10 cm.

The hot blot method was used to demonstrate that each of the amplified sequences was specific. In the hot blot method a 5’ end-labelled oligonucleotide probe that is complementary to the amplified sequence for an interval internal to the primers is included in a final cycle of PCR. Therefore, a band
corresponding to a particular amplified product will
only show up on the hot blot if that product contains
sequences that are complementary to the PCR primers
to allow amplification and if the product contains the
internal sequence that is complementary to the oligo-
nucleotide probe included in the hot blot reaction.
The specifically labelled PCR products are resolved on
an agarose gel. The gel is dried and autoradiography is
performed with OMAT-AR (Kodak, New Haven, CT)
film with an intensifying screen. The hot blot is faster
and eliminates a large proportion of the high back-
ground that is noted during Southern blotting of PCR
products. This method increases the sensitivity of de-
tection of probe-specific PCR amplification products
compared with visualization of bands on an ethidium-
bromide-stained gel and demonstrates the specificity
of the amplification product of the expected size. The
internal FGF receptor-1 probe for hot blotting was
designed from the published sequence for FGF recep-
tor-1 cDNA16 (inverse complement of nucleotides
1084-1113, sequence of probe 5'-GGA-GAC-ATT-
TCT-TAA-GTG-AAG-CAC-CTC-CAT). The FGF re-
ceptor-1 internal probe was designed so that each of
the PCR amplification products corresponding to the
three amino-terminal motifs of FGF receptor-1 pro-
tein would be identified.10 This probe has been shown
to identify sequences coding for the three amino-ter-
minal motifs of FGF receptor-1 in corneal tissues.17
The internal interleukin-1 receptor probe was de-
designed from the published sequence11 (inverse com-
plement of nucleotides 4529-4558 (sequence of probe
5' to 3' TGC-AAG-AAA-ATC-TAA-TTA-ATG-TGG-
AAG-GCA). The internal glucocorticoid receptor
probe was designed from the published sequence12
(inverse complement of nucleotides 1798-1827, se-
quence of probe 5'-GTT-GAG-CGT-AGT-
CAT-GAT-CCT-CCA-AGT).

Results
Figure 1 shows an ethidium bromide–stained gel with
PCR amplification products for beta actin and inter-
leukin-1 receptor from the three human lacrimal
gland samples. Each sample yielded a strong amplifi-
cation band for the beta actin control, demonstrating
that each cDNA sample was of sufficient quality to
detect a relatively abundant messenger RNA se-
quence. No genomic beta actin amplification products
were detected demonstrating that the cDNA samples
were free of genomic DNA contamination. Interleu-
klin-1 receptor amplification products of the expected
size of 397 base pairs were also easily detected in each
lacrimal gland sample on the ethidium-bromide-
stained gel.

Amplification products of the expected size for
the glucocorticoid receptor and the beta amino-ter-
minal motif of FGF receptor-1 were detected in each
sample by visual inspection of the ethidium-bromide-
stained gel, but bands were faint and difficult to see on
photographic reproductions. Neither alpha nor
gamma amino-terminal motif FGF receptor-1 amplifi-
cation products were detected in any of the lacrimal
samples.

Figure 2 shows hot blots for the IL-1 receptor and
glucocorticoid receptor. Specific amplification prod-
ucts of the expected size of 643 base pairs for the

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933399/)

**Figure 1.** Ethidium bromide–stained agarose gel of polymerase chain reaction amplification products for interleukin-1 receptor and beta actin. Lane M is the 100 base-pair DNA ladder with sizes marked in base pairs to the left. Columns marked CE are amplification products from corneal epithelial cDNA as a positive control. Amplification products of the expected size for interleukin-1 receptor and beta actin were detected in each sample of human lacrimal gland tissue. Amplification products of the expected size for fibroblast growth factor receptor-1 and glucocorticoid receptor from the lacrimal samples appeared faint when stained with ethidium bromide gel. Although the expected polymerase chain reaction product for each receptor was detectable in each lacrimal sample, they were not easily seen on photographic reproductions and are not shown.
FIGURE 2. Hot blots of polymerase chain reaction amplification products for the glucocorticoid receptor (A) and interleukin-1 receptor (B). Specific amplification products of the expected size (643 base pairs, large arrowhead) for the glucocorticoid receptor (GluR) were detected in each human lacrimal gland sample. Specific amplification products for the interleukin-1 receptor of the expected size of 397 base pairs (arrow) were also detected in each human lacrimal gland sample. In addition, an alternative product approximately 290 base pairs in size (small arrowhead) was also present.

glucocorticoid receptor were detected in each lacrimal gland cDNA sample. IL-1 receptor amplification products of the expected size of 397 base pairs were also detected in each lacrimal gland sample. In addition, an alternative product approximately 290 base pairs in size was also detected (Fig. 2).

Figure 3 shows hot blots for FGF receptor-1 amplification from lacrimal gland and primary corneal epithelial cell cDNA. The specific 800 base-pair amplification product for the beta amino-terminal motif of FGF receptor-1 was detected in each human lacrimal gland sample. Amplification products for the alpha and gamma amino-terminal motifs were not detected despite overexposure of the hot blot for 2 weeks longer than the blot shown in Figure 3 and the detection of these motifs in corneal epithelial cells tested simultaneously.

Discussion

The polymerase chain reaction and hot blotting were used to demonstrate that messenger RNAs coding for FGF receptor-1, IL-1 receptor, and glucocorticoid receptor are produced by cells within human lacrimal tissue. A smaller alternative amplification product for the IL-1 receptor was also detected in human lacrimal tissue. It is likely that this alternative product was generated in the lacrimal tissue through alternative mRNA splicing, but its function is unknown.

Messenger RNA molecules specify the sequence of the corresponding protein during protein synthesis. The highest order of regulation of protein synthesis is usually at the level of messenger RNA synthesis and, therefore, identification of FGF receptor-1, IL-1 receptor, and glucocorticoid receptor mRNA molecules provides strong evidence that the corresponding proteins are produced in human lacrimal tissue. Immunoblotting methods should, however, be used to confirm that FGF receptor-1, IL-1 receptor, and glucocorticoid receptor proteins are translated from the mRNA in this tissue.

As with the previously described synthesis of EGF, EGF receptor, and basic FGF in lacrimal tissue, this study does not establish which cells in the lacrimal gland, including possibly blood cells, produce FGF receptor-1, IL-1 receptor, and glucocorticoid receptor messenger RNAs. In situ hybridization methods are needed to identify the specific cell or cells that participate in the production of each of these mRNA.

Three members of the FGF receptor family [FGF receptor-1 (Flg-encoded), FGF receptor-2 (bek-encoded), and FGF receptor-3] have been reported. The PCR has been used in the present study to demonstrate that mRNA coding for FGF receptor-1 is pres-
FGF Receptor-1, IL-1 Receptor, and Glucocorticoid Receptor in Lacrimal Gland

Corneal Epithelium

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FIGURE 3. Hot blots of polymerase chain reaction amplification products for fibroblast growth factor receptor-1 in human primary corneal epithelial cells and lacrimal tissue. Only the 800 base-pair amplification product for the beta amino-terminal motif of fibroblast growth factor receptor-1 (large arrowhead) was detected in each human lacrimal gland sample. Amplification products for the alpha (1100 base pairs) and gamma (1000 base pairs) amino-terminal motifs were not detected in lacrimal tissue, despite exposure of the hot blot for 2 weeks longer than the blot shown and detection of mRNA for all three amino-terminal motifs in the primary corneal epithelial cells.

ent in human lacrimal gland. Only mRNA coding for the beta amino-terminal motif of FGF receptor-1 was detected in lacrimal tissue. This motif contains two extracellular IgG-like disulfide loops and is thought to bind basic FGF exposed to the cell surface. The alpha amino-terminal motif of FGF receptor-1 that contains three extracellular IgG-like disulfide loops and the gamma amino-terminal motif of FGF receptor-1 was not detected in human lacrimal tissue. The gamma motif does not contain a known signal sequence for membrane translocation is believed to represent an intracellular form of FGF receptor-1. Studies of the effect of basic FGF on lacrimal cells could help to determine the function of the beta amino-terminal motif of FGF receptor-1 because the effects regulated by this motif can be examined without interference from the alpha and gamma amino-terminal motifs present in other tissues such as corneal epithelial and endothelial cells.

The functions regulated by FGF receptor-1, IL-1 receptor, and glucocorticoid receptor in the lacrimal gland remain to be elucidated. The identification of FGF receptor-1 mRNA, along with the previous identification of basic FGF mRNA in lacrimal tissue, suggests an autocrine and/or paracrine role for basic FGF in the lacrimal gland. This does not, however, rule out the possibility that lacrimal gland-produced basic FGF could be released into tears and modulate functions of the cells of the ocular surface. Because basic FGF does not have a classic secretory sequence, however, mechanisms through which the basic FGF could be released from lacrimal cells remain unknown. More studies with isolated lacrimal cells must be done to further delineate the functions of these growth factors, cytokines, and receptors in lacrimal gland physiology.

Key Words
lacrimal gland, fibroblast growth factor receptor-1, interleukin-1 receptor, glucocorticoid receptor, polymerase chain reaction

References


13. Mivechi NF and Rosi JJ. Use of polymerase chain reaction to detect the expression of the Mr 70,000 heat shock genes in control or heat shock leukemic cells as correlated to their heat response. *Cancer Res.* 1990;50:2877–2884.


