SECRETORY PEPTIDES TFF1 AND TFF3 SYNTHESIZED IN HUMAN CONJUNCTIVAL GOBLET CELLS

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PURPOSE. The objective of this study was to determine whether human conjunctival epithelium synthesizes TFF-peptides (formerly P-domain peptides, trefoil factors), a family of mucin-associated secretory peptides of the gastrointestinal tract.

METHODS. Expression of TFF-peptides in human conjunctiva was monitored by reverse transcription-polymerase chain reaction analysis. Antisera specific for TFF-peptides were used for immunohistochemistry to determine the presence and distribution of TFF-peptides in human conjunctiva.

RESULTS. mRNA expression of TFF1 and TFF3, but not TFF2, was detected in human conjunctiva. TFF1 and TFF3, but not TFF2, are stored in conjunctival goblet cells only as revealed by immunofluorescence.

CONCLUSIONS. Goblet cells of the human conjunctiva synthesize TFF1 and TFF3. These peptides, together with the secretory ocular mucin MUC5AC, may contribute to the rheological properties of the tear film. They also may influence healing of corneal wounds due to their motogenic properties. (Invest Ophthalmol Vis Sci. 1999;40:2220–2224)

The surface of the eye is overlaid by a complex tear film, which contributes optical clarity, lubrication, and a protective barrier against pathogenic and noxious agents. This film is approximately 35- to 45-μm thick and is composed of three layers: an outer lipid layer, secreted by the meibomian glands; an intermediate aqueous layer, secreted mainly by the lacrimal glands; and an inner mucus layer of approximately 30 μm containing mucins as its major structural component.1,2

Mucins influence the rheological properties of the ocular mucus.1,2 The rheological properties are defined by the tear break-up time, which is changed in various pathologic conditions (e.g., in patients with dry eye symptoms). Alterations of mucin in human conjunctival epithelia of such patients have been reported.

TFF-peptides (formerly P-domain peptides, trefoil factors) like mucins are typical constituents of mucus gels (e.g., from the gastrointestinal9–12 and the respiratory13 tracts), and amphibian integumentary mucins contain integral TFF-domains.14 Three TFF-peptides are known to exist in humans: TFF1 (formerly pS2), TFF2 (formerly hSP), and TFF3 (formerly hP1.B/hITF). They show distinct expression patterns; however, they all are typically secreted by mucin-producing cells (e.g., several types of goblet cells). TFF-peptides are thought to modulate the rheological properties of mucus gels by specific interaction with mucins.15,16 Furthermore, all three TFF-peptides are motogens that influence the migration rates of cell lines in wound healing assays in vitro17–19 and increase the resistance of animals against gastrointestinal damage in vivo.20–24 This makes them candidates as factors regulating rapid repair of mucous epithelia by a process called restitution.8

TFF3 has recently been shown to be accumulated in goblet cells of porcine conjunctival epithelia, perhaps serving as a protective agent.25 An analysis of TFF-peptides, expanded to the expression of all three members, in human conjunctival epithelial tissues is presented here.

MATERIALS AND METHODS

Antisera

The following antisera-monitoring TFF-peptides were used:

Anti-TFF1, a monoclonal mouse antiserum against the 30 C-terminal amino acids of human TFF1, was purchased from

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Human conjunctiva bulbi tissues were obtained in the course of cataract surgery (3 men, 1 woman; 70 to 85 years of age) or strabismus surgery (4 females; 6 to 31 years of age). None of the patients were using extended preoperative eye medication except one patient receiving topical eye medication for glaucoma. All conjunctiva obtained appeared normal at the time of preoperative examination. Freshly excised conjunctiva bulbi tissue was immediately frozen in liquid nitrogen, and RNA was extracted from the pooled tissue samples using a guanidinium thiocyanate protocol. RNA purification via CsCl ultracentrifugation and reverse transcription–polymerase chain reaction (RT–PCR) analysis monitoring expression of TFF1, TFF2, and TFF3 were essentially as described previously, with 30 amplification cycles (Taq DNA polymerase; Boehringer Mannheim, Mannheim, Germany). As a control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts were amplified in a parallel reaction using a specific primer pair.

General Histology and Immunofluorescence

Human conjunctiva bulbi tissues were obtained in the course of cataract surgery (2 men, 4 women; 65 to 88 years of age) for which informed consent was obtained from the subjects. All patients were free of preoperative chronic medication except one patient receiving topical eye medication for glaucoma. All conjunctiva obtained appeared normal at the time of preoperative examination. Freshly excised conjunctiva bulbi tissue was immediately frozen in liquid nitrogen, and RNA was extracted from the pooled tissue samples using a guanidinium thiocyanate protocol. RNA purification via CsCl ultracentrifugation and reverse transcription–polymerase chain reaction (RT–PCR) analysis monitoring expression of TFF1, TFF2, and TFF3 were essentially as described previously, with 30 amplification cycles (Taq DNA polymerase; Boehringer Mannheim GmbH, Mannheim, Germany). As a control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts were amplified in a parallel reaction using a specific primer pair.

RESULTS

RT–PCR Analysis

RNA was isolated from pooled conjunctiva tissue of six patients of which cDNA was amplified by the use of specific primer pairs, testing for TFF1, TFF2, or TFF3 transcripts (Fig. 1A). TFF1- and TFF3-specific amplification products were clearly visible after separation on an agarose gel. In contrast, expression of TFF2 was not detectable in human conjunctiva. As controls, TFF1, TFF2, and TFF3 transcripts were monitored in parallel reactions with cDNA samples from human stomach and colon, respectively (Fig. 1B).

Immunofluorescence Analysis

The cellular localization of TFF1 and TFF3 revealed a similar pattern. Both peptides are stored exclusively in secretory granules of conjunctival goblet cells, which are Alcian blue-positive due to their characteristic mucin contents (Fig. 2). However, there are variations in the intensity between different goblet cells concerning their relative amounts of TFF1 and TFF3 (Fig. 3). Staining for TFF1 and TFF3 is shown to be specific because it could be competitively inhibited by the corresponding peptides in parallel sections (Figs. 2E, 2F). TFF2 could not be detected in all the conjunctiva samples tested

FIGURE 1. RT–PCR. (A) TFF1, TFF2, and TFF3 expression was monitored from human conjunctiva RNA pooled from six different patients (lanes a, b, c, d). The integrity of the cDNA was tested by amplification of the GAPDH transcript (lane a). (B) Total RNA from human stomach (lanes a, b, c) or colon (lane d) was analyzed as positive controls for GAPDH, TFF1, TFF2, or TFF3 transcripts, respectively.
FIGURE 2. TFF1 and TFF3 in human conjunctival epithelium. (A) Localization of TFF1 to goblet cells using the monoclonal TFF1 antiserum and immunofluorescence with Cy3-label; counterstaining was with DAPI. (B) Localization of TFF3 to goblet cells using antiserum anti-rTFF3-1 and immunofluorescence with fluorescein-label; counterstaining was with DAPI. (C, D) Phase-contrast pictures of parallel sections to (A) and (B), respectively, stained with periodic acid–Schiff/Alcian blue. (E, F) No staining was observed in parallel reactions to (A) and (B) after competition with recombinant TFF1/dimer (E) or the synthetic peptide FKPLQEAECTF (F), respectively. Scale bars, 30 μm.

FIGURE 3. Sequential colocalization of TFF1 and TFF3 in human conjunctival goblet cells. (A) Localization of TFF1 using the monoclonal antiserum and immunofluorescence with Cy3-label after (C) having localized first TFF3 with anti-rTFF3-1 and fluorescein label. (B) Phase-contrast picture of (A, C). Scale bars, 30 μm.
Respiratory submucosal glands TFF3/MUC5B
Respiratory goblet cell TFF3/MUC5AC
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the rheological properties of mucous gels.15 This hypothesis
respiratory goblet cells. 13,15,30 Thus, the localization of
in gastric surface cells,27–29 whereas TFF3 is found in intestinal
and respiratory goblet cells.13,15,30 Thus, the localization of
 Conjunctival goblet cells matches precisely that of the secretory mucin
tissue of the secretory peptides TFF1 and TFF3, but not TFF2, in
the human conjunctival epithelium. This result is in agreement
with a similar observation concerning TFF3 in porcine materi-
al.25 Both TFF1 and TFF3 are localized in the same conjunctival
goblet cells, which does not conform to most other mucous
epithelium that show a nonoverlapping distribution pattern of
TFF-peptides (Table 1). For example, TFF1 is typically localized
in gastric surface cells, whereas TFF3 is found in intestinal
and respiratory goblet cells.13,15,30 Thus, the localization of
TFF1 in conjunctival goblet cells is remarkable because one
would have expected its expression in apical cells of the
conjunctival epithelium (together with MUC4) by analogy with
its gastric expression.

The localization of TFF1 and TFF3 in the human conjunc-
tival goblet cells matches precisely that of the secretory mucin
MUC5AC.1,4,3 Thus, conjunctival TFF1 and TFF3 have to
be considered typical mucin-associated peptides. Note that each
mucin-producing cell type secretes a characteristic TFF-pep-
tide/mucin combination, probably reflecting the complex
physiological needs of the environment of these cells (Table 1).
In particular, the cocktail secreted by conjunctival goblet cells
is a combination of that secreted by gastric surface cells and
respiratory goblet cells.

It is postulated that TFF-peptides function as “link-pep-
tides” interacting noncovalently with mucins and influencing
the rheological properties of mucous gels.15 This hypothesis
has been confirmed in preliminary studies with TFF2 and TFF3,
both increasing the viscosities of mucin preparations.16 The
dimeric structure of TFF131 and TFF332 is ideally suited to form
an entangled network33,34 with MUC5AC in the ocular mucus.
The precise nature of the interaction between TFF-peptides
and mucins is currently not known; generally, protein–protein
and protein–sugar interactions are imaginable. However, the
latter has been proposed for TFF2 because of its established
three-dimensional structure.35

A second possible function of TFF1 and TFF3 in the eye is
that of motogens by analogy to their wound healing properties
in the gastrointestinal tract. After a wound in the cornea,
migration of surrounding corneal epithelial cells is observed,
whereas a total corneal epithelial defect can only be healed by
conjunctival epithelial cells covering the denuded cornea.36–38
A corneal wound stimulates goblet cell mucus secretion in the
same eye by activation of efferent parasympathetic and symp-
pathetic nerves in the rat conjunctiva.39,40 This reflex secre-
tion could also release TFF1 and TFF3.
Mucin-deficiency disorders41 such as ocular cicatricial pemphigoid, Steven-Johnson syndrome, or xerophthalmia
could lower levels of TFF-peptides parallel to the decrease in
goblet cell densities. In contrast, a higher number of goblet
cells42 might enhance TFF-peptide secretion as when contact
lenses are worn daily. TFF-peptide secretion might also be
influenced by alterations in glycosylation of goblet cell mucins
as they occur in patients with dry eye symptoms.4 However, as
yet there are no data concerning synthesis and secretion of
ocular TFF-peptides in pathologic conditions.

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### Table 1. Predominant TFF-Peptide/Secretory Mucin Combinations Observed in Various Human Mucin-Producing Cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>TFF-Peptide/Mucin</th>
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<tbody>
<tr>
<td>Gastric surface cell</td>
<td>TFF1/MUC5AC</td>
</tr>
<tr>
<td>Gastric mucous neck cell</td>
<td>TFF2/MUC6</td>
</tr>
<tr>
<td>Intestinal goblet cell</td>
<td>TFF3/MUC2</td>
</tr>
<tr>
<td>Conjunctival goblet cell</td>
<td>TFF1 +3/MUC5AC</td>
</tr>
<tr>
<td>Respiratory goblet cell</td>
<td>TFF3/MUC5AC</td>
</tr>
<tr>
<td>Respiratory submucosal glands</td>
<td>TFF3/MUC5B</td>
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