Characterization of Mucins in Human Lacrimal Sac and Nasolacrimal Duct

Friedrich P. Paulsen,[1] *Anthony P. Corfield,*[2] *Margitta Hinz,*[3] *Werner Hoffmann,*[3] *Ulrich Schaudig,*[4] *Andreas B. Thale,*[5] *and Monica Berry*[6]

Purpose. Mucins are polymers that may reduce drag and enhance tear outflow. Mucin expression and distribution in human efferent tear ducts were tested in the physiological state, and potential differences in the expression pattern were investigated in the presence of primary acquired dacryostenosis (PANDO).

Methods. Expression of mucins in human lacrimal sac and nasolacrimal ducts was monitored by reverse transcription–polymerase chain reaction analysis. The presence and distribution of MUC1, -2, -4, -5AC, -5B, -6, and -7 in epithelia of the efferent tear duct passage are assessed with antisera to mucin peptide cores. Twenty normal tissues from cadavers and surgical specimens from 20 patients with PANDO were tested.

Results. mRNAs for all mucins investigated were detected in healthy human lacrimal sacs and nasolacrimal ducts. MUC6 mRNA was detected in only about half of the investigated samples. A reduced level of MUC2, -5AC, and -5B mRNAs was observed in PANDO. Immunohistochemistry revealed MUC2 in goblet cells and single epithelial cells. Both MUC5AC and -5B were detected in goblet cells forming intraepithelial mucous glands. MUC7 was present only in columnar epithelial cells of the efferent tear duct system. No immunoreactivity was observed with antibodies against MUC1, -4, and -6 peptide cores.

Conclusions. Human efferent tear ducts express and produce a broad spectrum of mucins that is partly comparable with that in the conjunctiva and the salivary glands. The mucin diversity of the efferent tear ducts could enhance tear transport and antimicrobial defense. Reduced levels of mucin mRNA in a nonfunctioning though patent segment of the lacrimal passage, which is associated with epiphora, suggests that mucins ease tear flow through the efferent tear ducts. (*Invest Ophthalmol Vis Sci.* 2003;44:1807–1813) DOI:10.1167/iovs.02-0744

Although the physiology of lacrimal drainage has been studied for more than a century, the forces that cause tear flow are not completely understood. Various mechanisms have been proposed to explain the drainage of tears. These include an active lacrimal pump mechanism, functioning by contraction of the orbicularis eye muscle; a "wrung out" mechanism, governed by a system of helically arranged fibrillar structures; and the action of a cavernous body surrounding the lacrimal sac and the nasolacrimal duct. Physical factors, such as capillarity, gravity, respiration, evaporation, and reabsorption of tear fluid through the lining epithelium of the efferent tear ducts have also been invoked (for reviews, see Refs. 1,2).

The presence of mucins in the efferent tear ducts has been suggested by several anatomic and biochemical facts. The lining epithelium of the lacrimal sac and the nasolacrimal duct is a pseudostratified, columnar epithelium rich in goblet cells. The goblet cells are integrated in the epithelium as solitary cells or, particularly in the lacrimal sac, in a characteristic arrangement of several cell groups.[3–5] The secretions of the epithelial cells have been shown to contain several carbohydrates consisting of galactose, *N*-acetyl-glucosamine, fucose, O-acetylated, and non-O-acetylated sialic acids[3] as well as the trefoil factor family (TFF) peptides TFF1 and -3.[5] The latter are intimately associated with mucins (e.g., the conjunctival MUC5AC is colocalized with TFF1 and -3).[6,7]

The high impact of mucins on the integrity of mucous epithelia and on the rheologic and antimicrobial properties of the tear film led us to a detailed analysis of their expression and distribution in human tear duct epithelial cells in the healthy state and to investigate potential differences in the presence of primary acquired dacryostenosis (synonymous with primary acquired nasolacrimal duct obstruction; PANDO).

Materials and Methods

The lacrimal systems from 20 cadavers (5 male, 15 female, aged 55–96 years) and 20 biopsy specimens obtained from patients at dacryocystorhinostomy (6 men, 14 women, aged 33–86 years) were analyzed. Surgical material was obtained with the permission of the Medical Ethics Committee and used in accordance with the Declaration of Helsinki.

Collection of Human Efferent Tear Duct Samples from Cadavers

Donor cadavers were free of recent trauma, eye or nasal infections, or disease involving or affecting lacrimal function. Dissection of the bony canal containing the lacrimal sac and nasolacrimal duct was performed less than 24 hours after death. The soft tissue representing right and left lacrimal sacs and nasolacrimal ducts was prepared. The 20 right efferent tear duct systems were frozen in liquid nitrogen. The 20 left efferent tear duct systems were fixed in 4% formalin, dehydrated, and embedded in paraffin.

Patients

Patients were selected from referrals to the Department of Ophthalmology, Christian Albrecht University, Kiel, Germany, between May 1999 and May 2001 or the Department of Ophthalmology, University Hospital Hamburg-Eppendorf, Germany, between August 1999 and June 2001. Of 77 patients with epiphora, 20 had functional obstruction with a patent lacrimal system on syringing and were included in the study. These 20 patients had epiphora of between 5 weeks and 16

From the [1]Institute of Anatomy and [5]Eye Clinic, Christian-Albrechts University, Kiel, Germany; the Departments of [2]Medical Laboratories and [6]Ophthalmology, University of Bristol, United Kingdom; the [3]Institute for Molecular Biology and Medical Chemistry, Otto-von-Guericke University, Magdeburg, Germany; and the [4]Clinic for Ophthalmology, University Hospital Eppendorf, Hamburg, Germany.

Supported by German Research Foundation Grants Pa 738/1-2 and 738/1-3 (FPP) and Grant 2000/013 from the National Eye Research Center (MB).

Submitted for publication July 23, 2002; revised September 5, September 22, and October 31, 2002; accepted December 5, 2002.

Disclosure: **F.P. Paulsen**, None; **A.P. Corfield**, None; **M. Hinz**, None; **W. Hoffmann**, None; **U. Schaudig**, None; **A.B. Thale**, None; **M. Berry**, None

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Corresponding author: Friedrich P. Paulsen, Anatomisches Institut der Christian-Albrechts-Universität zu Kiel, Olshausenstrasse 40, D-24098 Kiel, Germany; fpaulsen@anat.uni-kiel.de.

TABLE 1. Primers Used for RT-PCR

Mucin	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Length (bp)
MUC1	GGT ACC TCC TCT CAC CTC CTC CAA	CGT CGT GGA CAT TGA TGG TAC C	287
MUC2	ACT GCA CAT TCT TCA GCT GC	ATT CAT GAG GAC GGT CTT GG	233
MUC4	TTC TAA GAA CCA CCA GAC TCA GAG C	GAG ACA CAC CTG GAG AGA ATG AGC	467
MUC5AC	CCA AGG AGA ACC TCC CAT AT	CCA AGC GTC ATT CCT GAG	282
MUC5B	CTG CGA GAC CGA GGT CAA CAT C	TGG GCA GCA GGA GCA CGG AG	415
MUC6	CAG CAG GAG GAG ATC ACG TTC AAG	GTG GGT GTT TTC CTG TCT GTC ATC	324
MUC7	CTT CTC GTT CAG TGA AGG TCG	TGG AAG CTT AGG CCT ACA GC	178
GAPDH	CCA GCC GAG CCA CAT CGC TC	ATG AGC CCC AGC CTT CTC CAT	983

months duration. Fifty-seven patients were excluded. Exclusion criteria were: facial surgery or trauma, allergies, family history of tearing, external eyelid disease, topical eye medication, eyelid malposition, periocular neoplasm, punctal or canalicular stenosis, acute dacryocystitis, sinusitis, nasal disease, or complete occlusion of the lacrimal passage.

Dacryocystorhinostomy was performed in all 20 selected patients. Of these, 5 had stenosis of the lacrimal sac, and 15 had stenosis that was localized in the nasolacrimal duct. Biopsy specimens were always taken at the site of the surgical opening of the lacrimal sac (i.e., from the center of the lacrimal sac) and frozen in liquid nitrogen.

Total RNA Purification and cDNA Synthesis

For reverse transcription–polymerase chain reaction (RT-PCR), frozen samples were crushed in an agate mortar under liquid nitrogen, insoluble material was removed by centrifugation (12 000*g*, 5 minutes, 4°C), and RNA was isolated as described by the manufacturer (by the phenol-guanidinium thiocyanate method). Crude RNA was purified by isopropanol and repeated ethanol precipitation, and contaminating DNA was destroyed by digestion with RNase-free DNase I (27.27 Kunitz units; 20 minutes, 25°C; Roche Molecular Biochemicals, Mannheim, Germany). RNA quantity was measured photometrically, so that equal amounts were loaded (500 ng). After inactivation, the DNase (15 minutes, 65°C), cDNA was generated with 50 ng/μL (20 pmol) oligo $(dT)_{15}$ primer (Amersham Pharmacia Biotech, Uppsala, Sweden) and 0.8 μL reverse transcriptase (100 U Superscript RNase H^-; Gibco, Paisley, UK) for 60 minutes at 37°C. The integrity of the RNA was controlled by RT-PCR of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with intron-spanning primers. No PCR products larger than 983 bp were detected that would have indicated contaminating DNA.

Reverse Transcription–Polymerase Chain Reaction

RT-PCR was performed in both surgically obtained and cadaveric tissues. For RT-PCR, 4 μL cDNA was incubated with 30.5 μL water, 4 μL 25 mM $MgCl_2$, 1 μL dNTP, 5 μL 10× PCR buffer, 0.5 μL (2.5 U) platinum *Taq* DNA polymerase (Gibco), and 2.5 μL (10 pmol) primers (Table 1). All PCR primer pairs for MUC genes were designed to amplify the 3′ non–tandem-repeat portion of the mRNA. The PCR cycle consisted of (1) 94°C for 30 seconds; (2) 64°C (MUC1), 57°C (MUC2), 64°C (MUC4), 57°C (MUC5AC), 64.7°C (MUC5B), 64°C (MUC6), and 60°C (MUC7) for 30 seconds each; (3) 72°C for 90 seconds; 35 cycles were performed with each primer pair.[8] The final cycle consisted of 72°C for 10 minutes; 0.1 μM of a GADPH intron-spanning primer pair served as the internal control for cDNA (Table 1). All primers were synthesized by MWG-Biotech AG, Ebersberg, Germany.

Positive controls included human conjunctiva (MUC1, -4, and -5AC) obtained from the Kiel Eye Bank, Kiel, Germany; small intestine (MUC2) and stomach (MUC5AC and -6), both obtained from the Department of Surgery, Christian Albrecht University; and cadaveric submandibular gland (MUC1, -5B, and -7) obtained from the Institute of Anatomy, Kiel, Germany. The cDNA was replaced with water for a negative control reaction.

Immunohistochemistry

Reactivity with a panel of antibodies against mucins (Table 2) was followed by immunohistochemistry in cadaveric tissue sections from 20 left lacrimal sac specimens and 20 left nasolacrimal duct specimens. All antibodies were tested with microwave heating pretreatment for 10 minutes, with periodate pretreatment for 30 minutes (periodate: 1.93 g ammonium acetate in 500 mL aqua dest

TABLE 2. Antibodies Used for Immunohistochemical Staining in Paraffin-Embedded Sections

Mucin	Antibody	Species	Dilution	Pretreatment	Peptide Specificity	Origin/Original Reference
MUC1	BC2	Mouse	1:1000	Microwave	VNTR	Xing et al.[9]
MUC1	NCL-MUC-1	Mouse	1:100	Microwave	VNTR	Novocastra, Newcastle-upon-Tyne, UK
MUC1	SM3	Mouse	1:50	Microwave	VNTR	Burchell et al.[10]
MUC1	HMFG1	Mouse	1:50	Microwave	VNTR	Taylor-Papadimitriou et al.[11]
MUC1	HMFG2	Mouse	1:50	Microwave	VNTR	Burchell and Taylor-Papadimitriou[12]
MUC2	NCL-MUC-2	Mouse	1:150	Microwave	VNTR	Novocastra
MUC2	MCA1743	Mouse	1:150	Microwave	VNTR	Serotec, Oxford, UK
MUC4	4F12	Mouse	1:50	Microwave	ASGP2	Developmental Studies Hybridoma Bank, Iowa City, IA
MUC5AC	NCL-MUC-5AC	Mouse	1:50	Microwave only or with periodate*	VNTR	Novocastra
MUC5B	LUM 5B-2	Rabbit	1:100	Microwave and periodate	non-VNTR	Wickström et al.[13]
MUC5B	5B III	Rabbit	1:100	Microwave and periodate	non-VNTR	Thomton et al.[14]
MUC6	LUM 6-3	Rabbit	1:100	Microwave	non-VNTR	Lopez-Ferrer et al.[15]
MUC6	NCL-MUC-6	Mouse	1:100	Microwave	VNTR	Novocastra
MUC7	LUM 7-1	Rabbit	1:100	Microwave	non-VNTR	Wickström et al.[16]

* The antibody against MUC5AC worked with and without periodate pretreatment but was more sensitive with pretreatment.

at pH 5 and 2.28 g periodic acidin), and without pretreatment. Appropriate pretreatment was as detailed in Table 2. All primary antibodies were applied overnight at room temperature. Secondary antibodies were rabbit anti-mouse (1:200; Dako, Glostrup, Denmark) for mouse-species–derived antibodies or swine anti-rabbit (1:300; Dako) for rabbit-species–derived antibodies applied at room temperature for at least 4 hours. Visualization was achieved by incubation with peroxidase-labeled streptavidin-biotin or diaminobenzidine (DAB) for at least 5 minutes. After they were counterstained with hemalum or nuclear fast red, the sections were mounted in aqueous medium (Aquatex; Roche Molecular Biochemicals). Two negative control sections were used in each case: One was incubated with the secondary antibody only, the other with the primary antibody only. Positive controls were as described in the RT-PCR section. All slides were examined by microscope (Axiophot, Carl Zeiss, Oberkochen, Germany).

Results

We used cadaveric tissue to study normal mucin expression and mucin distribution. Results from healthy tissue were compared with RT-PCR results from tissue affected by PANDO.

RT-PCR Analysis

Cadaveric Tissue. MUC1-specific amplification products (Fig. 1; Table 3) were visible in all nasolacrimal duct (Fig. 1, ND) samples and in 17 lacrimal sac (Fig. 1, LS) samples. Three LS samples were negative. MUC2-specific amplification products were clearly detected in every sample (Fig. 1; Table 3). MUC4 mRNAs were visible in all ND samples and in 15 LS samples. MUC5AC- and -5B–specific amplification products were detected in every sample except one (Fig. 1, lane 6). MUC6-specific amplification products were visible in nearly half of the investigated specimens; MUC7 mRNA was present in every sample (Fig. 1; Table 3). Positive controls of conjunctiva, small intestine, and submandibular gland samples yielded appropriate PCR products (not shown). Controls in nontranscribed RNA revealed lack of amplification (not shown).

Tissue from Patients with PANDO. Compared with healthy efferent tear duct systems, surgical specimens exhibited less expression of message for mucins, except the MUC4, -6, and -7 amplification products (Table 4), which showed no change. MUC1-specific mRNAs were absent in all but one patient with stenosis of the lacrimal sac. Nasolacrimal duct stenosis was associated with various expression levels of MUC1, revealing expression in nearly two thirds of the duct (Table 4). MUC2-, -5AC-, and -5B–specific amplification products were visible in a few samples from patients with nasolacrimal duct stenosis, and absent in all with lacrimal sac stenosis (Table 4). Positive controls of conjunctiva, small intestine, and submandibular gland samples indicated the methods were performed correctly (not shown).

Immunohistochemistry

Paraffin-embedded 7-μm sections from 20 normal (cadaveric) lacrimal sacs and 20 nasolacrimal ducts were analyzed. No differences in mucin distribution were seen between the lacrimal sac and the nasolacrimal duct. No reactivity was observed for MUC1 (Fig. 2A), with any of the five antibodies used (Table 2). MUC4 was not detected by antibody 4F12 (Fig. 2B), and no reactivity was observed with two different antibodies against MUC6 (Fig. 2C). Reactivity with anti-MUC2 antibodies was observed in single goblet cells (Fig. 2D) and intraepithelial mucous glands (Fig. 2E), as well as in single columnar epithelial cells (Fig. 2F). In the latter case, MUC2 showed a supranuclear granular distribution in the cytoplasm of the columnar cells. The presence of MUC2 in columnar epithelial cells is consistent with its expression in the absence of MUC5AC and -5B expression (Fig. 1, lane 6).

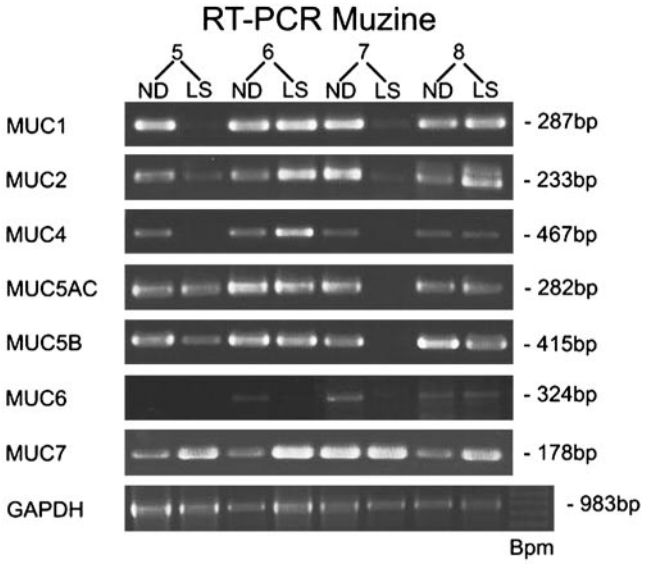

Figure 1. Expression of mucins in the epithelium of the human efferent tear system. RT-PCR analysis for MUC1, -2, -4, -5AC, -5B, -6, and -7 expression was performed in the epithelium of human efferent tear ducts of four cadavers (corresponding to cases 5–8 in Table 3). The integrity of the cDNAs was tested by amplification of the GAPDH transcript. Right: base pair standard. ND, nasolacrimal duct; LS, lacrimal sac.

Both MUC5AC (Fig. 2G) and -5B (Fig. 2H) were associated with single goblet cells and goblet cells forming intraepithelial mucous glands, but not columnar epithelial cells. MUC5AC revealed only weak reactivity, visible in granules of goblet cells, compared with the intense reactivity of the conjunctival goblet cells (Fig. 2M). Efferent tear ducts (Fig. 2H) were MUC5B positive (Table 2) after microwave and periodate pretreatments. Cytoplasmic reactivity with anti-MUC7 occurred in columnar epithelial cells of the lacrimal sac and the nasolacrimal duct (Fig. 2I).

The staining was confirmed by positive control sections: submandibular gland for MUC1 (Fig. 2J), -5AC (Fig. 2M), -5B (Fig. 2N), and -7 (Fig. 2P), small intestine for MUC2 (Fig. 2K), conjunctiva for MUC4 (Fig. 2L), and stomach for MUC6 (Fig. 2O).

Discussion

The human efferent tear ducts are integral parts of the lacrimal system and are involved in tear transport from the ocular surface to the inferior meatus of the nose. Proper functioning of the lacrimal sac and the nasolacrimal duct is reliant on the mucosa[3] and the underlying cavernous body,[17] which cover and surround both structures, clearing tear fluid from the ocular surface. Tear flow through the lacrimal sac and the nasolacrimal duct is largely dependent on interactions among mucosal mucins, microvilli, and the tear fluid coming from the ocular surface.

We have shown that the lacrimal sac and nasolacrimal duct have a different mucin gene expression pattern compared with the ocular surface. Conjunctiva mainly expresses MUC5AC, and, at much lower levels, MUC2.[18] In addition to these mucins, MUC7 is present in the tear film originating from the lacrimal gland.[19,20] Based on our immunohistochemical results, it appears that the epithelium of the lacrimal sac and nasolacrimal duct produce MUC5B and -7 and, to a lesser extent, MUC5AC and -2. There appears to be an alteration in the mucin pattern of the epithelium as it descends from the ocular surface

TABLE 3. Mucin mRNA Expression in Tissue Specimens from 20 Cadavers

Case	Age/Gender	SL	MUC1	MUC2	MUC4	MUC5AC	MUC5B	MUC6	MUC7	GAPDH
1	67/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+
2	81/F	LS	+	+	+	+	+	+	+	+
		ND	+	+	+	+	+	+	+	+
3	59/M	LS	+	+	+	+	+	+	+	+
		ND	+	+	+	+	+	+	+	+
4	73/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+
5	77/F	LS	−	+	−	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+
6	78/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	+	+	+
7	96/F	LS	+	+	−	−	−	−	+	+
		ND	+	+	+	+	+	+	+	+
8	64/F	LS	+	+	+	+	+	+	+	+
		ND	+	+	+	+	+	+	+	+
9	64/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+
10	76/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	+	+	+
11	87/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	+	+	+
12	55/M	LS	+	+	+	+	+	+	+	+
		ND	+	+	+	+	+	−	+	+
13	91/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	+	+	+
14	89/F	LS	−	+	−	+	+	+	+	+
		ND	+	+	+	+	+	+	+	+
15	60/M	LS	+	+	+	+	+	+	+	+
		ND	+	+	+	+	+	+	+	+
16	71/M	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+
17	66/F	LS	−	+	−	+	+	+	+	+
		ND	+	+	+	+	+	−	+	+
18	72/F	LS	+	+	−	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+
19	90/M	LS	+	+	+	+	+	+	+	+
		ND	+	+	+	+	+	+	+	+
20	83/F	LS	+	+	+	+	+	−	+	+
		ND	+	+	+	+	+	−	+	+

Cases 5–8 are demonstrated in Figure 1. SL, stenosis localization; LS, lacrimal sac; ND, nasolacrimal duct; F, female; M, male.

TABLE 4. Mucin mRNA Expression in Tissue Specimens from 20 Patients Who Have Undergone Endonasal Dacryocystorhinostomy in Primary Acquired Dacryostenosis

Case	Age/Gender	SL	D	MUC1	MUC2	MUC4	MUC5AC	MUC5B	MUC6	MUC7	GAPDH
1	35/F	ND	7 mo	+	−	++	−	−	−	+	+
2	78/F	ND	13 mo	+	−	+	−	+	−	+	+
3	59/F	ND	9 mo	−	−	+	−	−	−	+	+
4	47/M	LS	16 mo	−	−	+	−	−	−	+	+
5	83/F	ND	10 mo	−	−	−	−	−	−	+	+
6	58/F	ND	13 mo	−	−	+	−	−	+	+	+
7	80/F	ND	16 mo	+	−	+	−	+	+	+	+
8	53/F	LS	12 mo	−	−	+	−	−	+	+	+
9	77/F	LS	16 mo	−	−	+	−	−	−	+	+
10	71/F	ND	11 mo	+	+	+	−	+	−	+	+
11	57/F	ND	7 w	−	−	+	+	−	+	+	+
12	86/F	ND	14 mo	+	−	+	−	−	−	+	+
13	61/M	ND	12 mo	−	−	+	−	−	−	+	+
14	78/F	LS	15 mo	+	−	+	−	−	−	+	+
15	63/F	ND	11 mo	+	−	+	−	−	+	+	+
16	85/M	ND	15 mo	+	−	+	−	−	+	+	+
17	46/F	ND	5 w	+	−	+	+	−	+	+	+
18	71/F	LS	7 mo	−	−	+	−	−	−	+	+
19	38/M	ND	9 mo	+	−	+	−	−	−	+	+
20	33/M	ND	4 mo	+	+	+	+	+	−	+	+

SL, stenosis localization; LS, lacrimal sac; ND, nasolacrimal duct; D, duration of epiphora; F, female; M, male; mo, month; w, weeks.

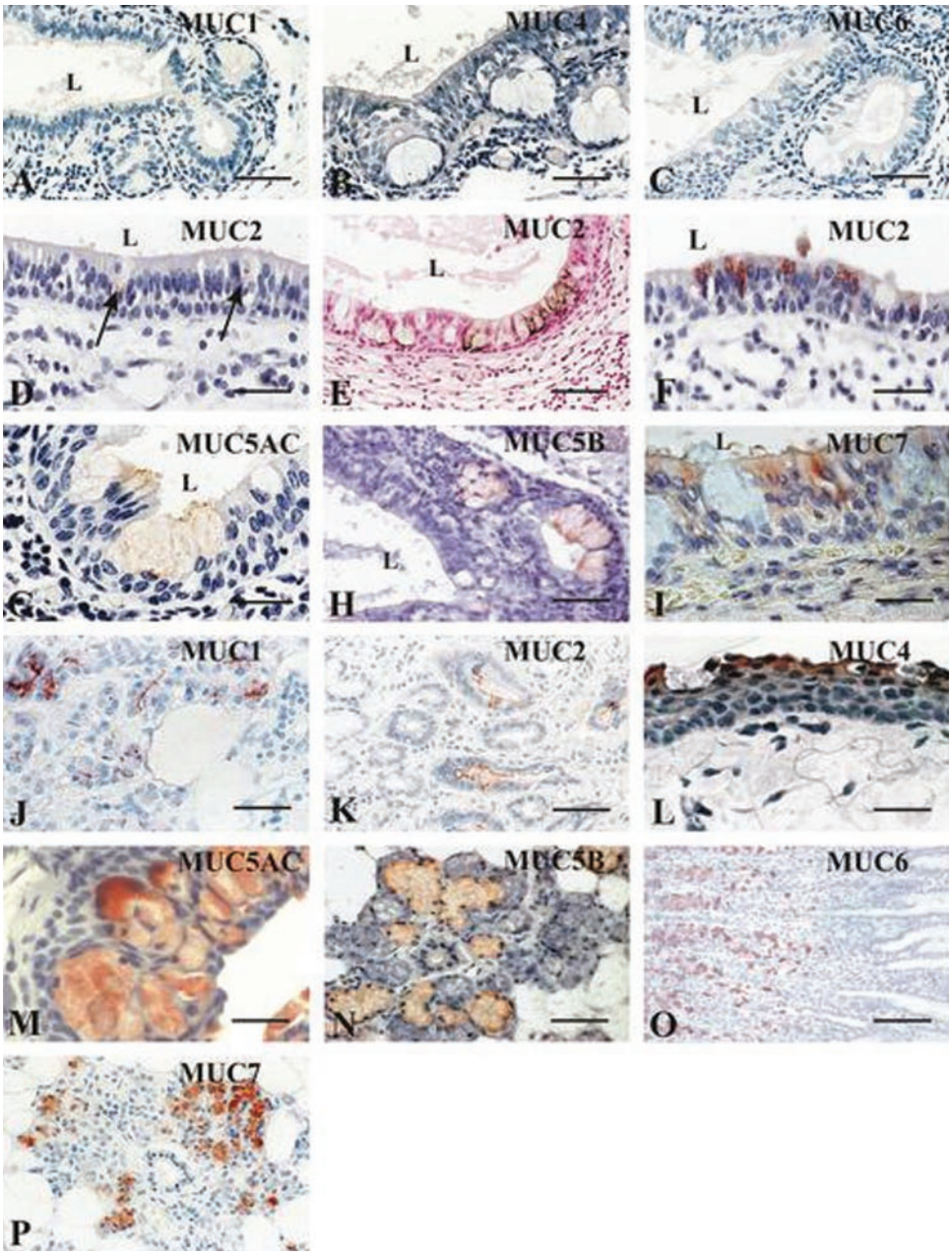

FIGURE 2. Distribution of mucins in human efferent tear ducts. No reactivity was demonstrated with antibodies (**A**) BC2 anti-MUC1 (one of the panel of five antibodies tested), (**B**) 4F12 against MUC4, or (**C**) LUM6-3 against MUC6 (selected example from two antibodies tested). The positive controls for these antibodies reacted as expected: (**J**) MUC1 (*red*) at the apical surface of serous cells of the submandibular gland; (**L**) MUC4 (*red*) in the superficial layers of conjunctival epithelium; and (**O**) MUC6 stomach (*red*) in mucous neck cells (LUM6-3). MUC2 was detected with (**D**) monoclonal antibody MCA1743 (*red*, *arrows*) in single goblet cells of the lacrimal sac; (**E**) monoclonal antibody NCL-MUC-2 (*brown*) in intraepithelial mucous glands of the lacrimal sac; and (**F**) MCA1743 (*red*) strong supranuclear granular staining in single columnar epithelial cells of the lacrimal sac. (**K**) Control section from the intestine revealing localization of MUC2 (*red*) in intestinal crypts (MCA1743). MUC5AC (**G**, *red*) was weakly reactive in intraepithelial mucous glands of the nasolacrimal duct, whereas conjunctival goblet cells were strongly reactive with this antibody (**M**). MUC5B (**H**, *red*) showed strong reactivity in intraepithelial mucous glands of the lacrimal sac. In the section from the submandibular gland control (**N**), MUC5B (*red*) reactivity was seen in mucous cells. In nasolacrimal ducts, cytoplasmic staining was observed in columnar epithelial cells probed for MUC7 (**I**). Control section from the submandibular gland showed MUC7 (*red*) in some serous cells (**P**). L, lumen of the efferent tear duct passage. Counterstains: (**A–D**, **F–P**) hemalum; (**E**) nuclear fast red. Scale bars: (**A**, **C**, **E**, **K**, **M**, **P**) 82.5 μm; (**D**, **F**, **J**) 56 μm; (**B**, **N**) 63 μm; (**G–I**, **L**) 38 μm; (**O**) 150 μm.

to the lacrimal sac. Expression and production of MUC5B were found in the epithelium of the efferent tear duct passage. MUC2 production also changed. We detected MUC2 in goblet cells and also in columnar epithelial cells (Fig. 2D), consistent with MUC2 expression in the specimen that showed absence of MUC5AC and -5B (Fig. 1, lane 6).

Goblet cells of efferent tear ducts synthesize and store the three 11p15 secretory mucins: MUC2, -5B, and -5AC. MUC6 mucin, however, was not detected, although message for this mucin was present in half the samples. The low-molecular-weight, non–gel-forming mucin MUC7 was localized in columnar epithelial cells in the efferent tear ducts, similar to its distribution in the submandibular gland, where MUC7 is the secretory product of salivary serous cells.[21] MUC7 has a lubricative[22,23] in addition to an antimicrobial function[24–26] in saliva and may have a similar role in the efferent tear ducts.

Membrane-bound mucins MUC1 and -4 were not detectable in the lacrimal sac and the nasolacrimal duct, although message could be detected by PCR in all nasolacrimal ducts, though not all lacrimal sacs. Comparable results were recently obtained by Lin et al.,[27] who were also unable to detect membrane bound mucins in the mucosa of the middle ear, although the mucosa of the auditory tube was positive for MUC1 and -4. An explanation for the negative immunohistochemistry would be that the antibodies used were raised to epitopes within the VNTR region of the peptide core. These antibodies are likely to have varying reactivities, depending on the glycosylation of the mucins in the tissues under study. Mature mucins, with dense glycosylation, may not react with antibodies directed toward tandem repeat epitopes of the peptide core. These regions support the maximal glycosylation density. If perinuclear reactivity is observed in these cases, it coincides with immature, lightly glycosylated, mucins still in the Golgi apparatus. Different glycosylation patterns may explain reactivity in the positive control samples.

A distinct regional distribution of secretory mucins in the lacrimal sac and nasolacrimal duct may significantly influence the rheology and thus the flow of tears through the efferent tear passage. At the ocular surface, mucin composition, distribution, and function are influenced by shear forces generated

during blinking (for review, see Ref. 28). In the efferent tear ducts, such forces are absent, and other mechanisms are necessary to ease the flow of tears. In this context, it would be of interest to know whether accumulation of one or more of these mucins in the lacrimal sac cavity is a major cause of dacryolith formation.

In common with all mucosae, the surfaces of lacrimal sac and the nasolacrimal duct are in constant contact with environmental microorganisms and hence are vulnerable to infection. Previous studies show that the efferent tear duct mucosa has developed a variety of anti-infection strategies to prevent colonization by microorganisms. These are required to thwart attacks from microorganisms leading to dacryocystitis, the most frequent disease of the efferent lacrimal system.[3,29–34] MUC5B and -7 should now be added to the antimicrobial mechanisms, as they have been shown to participate in bacterial adhesion.[24–26]

Idiopathic or primary acquired dacryostenosis, synonymous with primary acquired nasolacrimal duct obstruction (PANDO), is a syndrome of unknown etiology that accounts for most nontraumatic cases observed in adults. Pathologic investigations indicate that it results from fibrous obstruction secondary to chronic inflammation.[35–37] However, the pathophysiology of functional dacryostenosis (i.e., patients with epiphora despite patent lacrimal passages on syringing) is yet to be understood. The pathologic condition in the epithelia is characterized by squamous metaplasia with loss of goblet cells.[38] Consistent with the condition, a marked reduction of message for goblet-cell–associated mucins MUC2, -5AC, and -5B was observed in PANDO specimens. There were no changes in MUC7 expression (Table 4), despite metaplasia of the columnar epithelial cells. Unchanged production of the antimicrobial mucin MUC7 could help explain why dacryocystitis never develops in some patients with epiphora due to postsaccal stenosis.

A full understanding of the molecular function of mucins at the mucosal surface of the efferent tear duct passage will provide further insight into the occurrence of dacryocystitis, which often leads to residual functional impairment with epiphora. The factors controlling the production of efferent tear-duct–associated mucins are unknown, but it is possible that some infection risk factors such as old age, changes in hormonal status (postmenopausal women), or immunodeficiency are associated with a downregulation of mucin production. It has been suggested that the normally constant flow of tears ensures mucin production through a feedback control and that this production comes to a halt if tears are not drained into the nose.[1,2]

The diversity of mucins in the lacrimal sac and nasolacrimal duct can be linked with enhanced tear transport and antimicrobial defense. Reduced levels of mRNA of secretory mucins in functional dacryostenosis supports the assumption that mucins ease the tear flow through the efferent tear ducts.

Acknowledgments

The authors thank Karin Stengel for excellent technical assistance; Joy Burchell (Imperial Cancer Research Fund, London, UK) for SM3, HMFG1, and HMFG2; Ingemar Carlstedt (University of Lund, Lund, Sweden) for LUM5B-2, LUM6-3, and LUM7-1; Michael McGuckin (University of Queensland, Brisbane, Australia), for BC2; and David Thornton (University of Manchester, UK) for 5B III.

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E R R A T U M

Erratum in: In "Using Machine Learning Classifiers to Identify Glaucomatous Change Earlier in Standard Visual Fields" by Sample et al. (*Invest Ophthalmol Vis Sci.* 2002;43:2660–2665).

In this paper we reported that machine learning classifiers predicted conversion to abnormal visual fields on average 4 years earlier than traditional Statpac-like methods. When reviewing the data for inclusion in a new study, an error was discovered in the dates of conversion based on the Statpac-like methods. Correction of the error has reduced the reported superiority of the machine classifiers to identify change in visual fields earlier. The support vector machine with a Gaussian kernel (SVMg), the best performing classifier, now predicts visual field conversion a mean $\pm$ standard error of 0.40 $\pm$ 0.51 years earlier than the Statpac-like techniques. SVMg continued to show the best agreement with the presence of glaucomatous optic neuropathy at 94% (32/34) compared to 74% (25/34) for the Statpac-like methods.

This error was caused by a fault in Microsoft Excel. Excel stores dates as serial numbers that represent the number of days from the system date. In Excel for Windows, January 1, 1900 is the default system date. In Excel for Macintosh computers, it is January 1, 1904. When dates are copied from a file that uses the 1900 system to a file that uses the 1904 date system, the displayed dates will be 1462 days (4 years 1 day) later. The reverse happens on a Windows computer. Excel does not warn users of this potential error. One must be aware of the problem to seek information in the help file or to change the date system in Excel.