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The human false vocal folds – an analysis of antimicrobial defense mechanisms

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Abstract Inflammatory processes often lead to pathologic changes in the area of the larynx. A moistening function of the false vocal folds has been described frequently. Up to now we have little knowledge of the role of the false vocal folds in protection against pathogenic agents. The present study analyzes the structures of the false vocal folds in their relations to antimicrobial defense mechanisms. Investigations were performed on false vocal folds of larynges from 34 cadavers using histologic, histochemical and immunohistochemical methods. Seromucous glands, together with epithelial and goblet cells of the folds, synthesize a complex mucus layer. In all of the investigated samples this layer contains carbohydrates including *N*-acetyl-glucosamine, *N*-acetyl-galactosamine, galactose, mannose, fucose, and sialic acids. Furthermore, antimicrobial peptides like lactoferrin, lysozyme, alpha and beta defensins are also found in these structures. IgA, produced by plasma cells in the false vocal folds, is frequently integrated in the secretory product. Synthesized mucins, antimicrobial peptides and immunoglobulins form a specialized protective substance that is secreted mainly at the true vocal folds. Here the layer functions to lubricate the true vocal folds, resulting in positive functional consequences during vocal production. Moreover, together with immunocompetent cells, the protective layer seems to play a major role in antigen defense and prevents invasion of pathogenic agents.

Keywords Ventricular fold · Vestibular fold · Antimicrobial peptide · Natural peptide antibiotic · Mucin

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Introduction

Laryngeal airway infections, sometimes causing airway obstruction and life-threatening illnesses, are due to an imbalance between defense and attack mechanisms at the mucosal surface of the larynx (Millan and Cumming 1996). However, in view of the frequency of endotracheal intubations, laryngoscopies, bronchoscopies, and other invasive methods currently applied to the respiratory tract, it is astonishing that serious laryngeal inflammation is a rare event. In this context, little is known about the function of the false vocal folds, also known as the ventricular folds or vestibular folds.

The false vocal folds are two thick, sagittally oriented, individually sized mucosal duplications arising from the wall into the lumen of the supraglottic space and forming the medial wall of the laryngeal ventricle (ventricle of Morgagni; Tillmann and Wustrow 1982, Fig. 1A). The ventricular folds consist of mucous membranes, each enclosing a narrow band of approximately sixty mucous glands lodged in the submucous areolar tissue. The glands are of the tubuloacinar, mixed seromucous variety. Their excretory ducts open into the laryngeal ventricle or end at the rim of the false vocal folds. Normally covered by a double-layered columnar epithelium with integrated goblet cells, the false vocal folds are also physiologically lined by areas of non-stratified squamous epithelium. These areas multiply and enlarge with increasing age (Ruckes and Cause 1964; Tillmann and Wustrow 1982).

As early as 1871, Luschka (1871) presumed that the false vocal folds isolate the true vocal folds and facilitate their vibration. Moreover, Luschka (1871) suggested that the secretory product of the false vocal folds serves to lubricate the true vocal folds as well as moistening the breath.

The role of the false vocal folds in immune response is largely unclarified. Fränkel (1893) describes the frequent occurrence of lymph follicles in the wall of the false vocal folds, which he termed “the laryngeal tonsil.” Kracke et al. (1997) analyzed the distribution of orga-

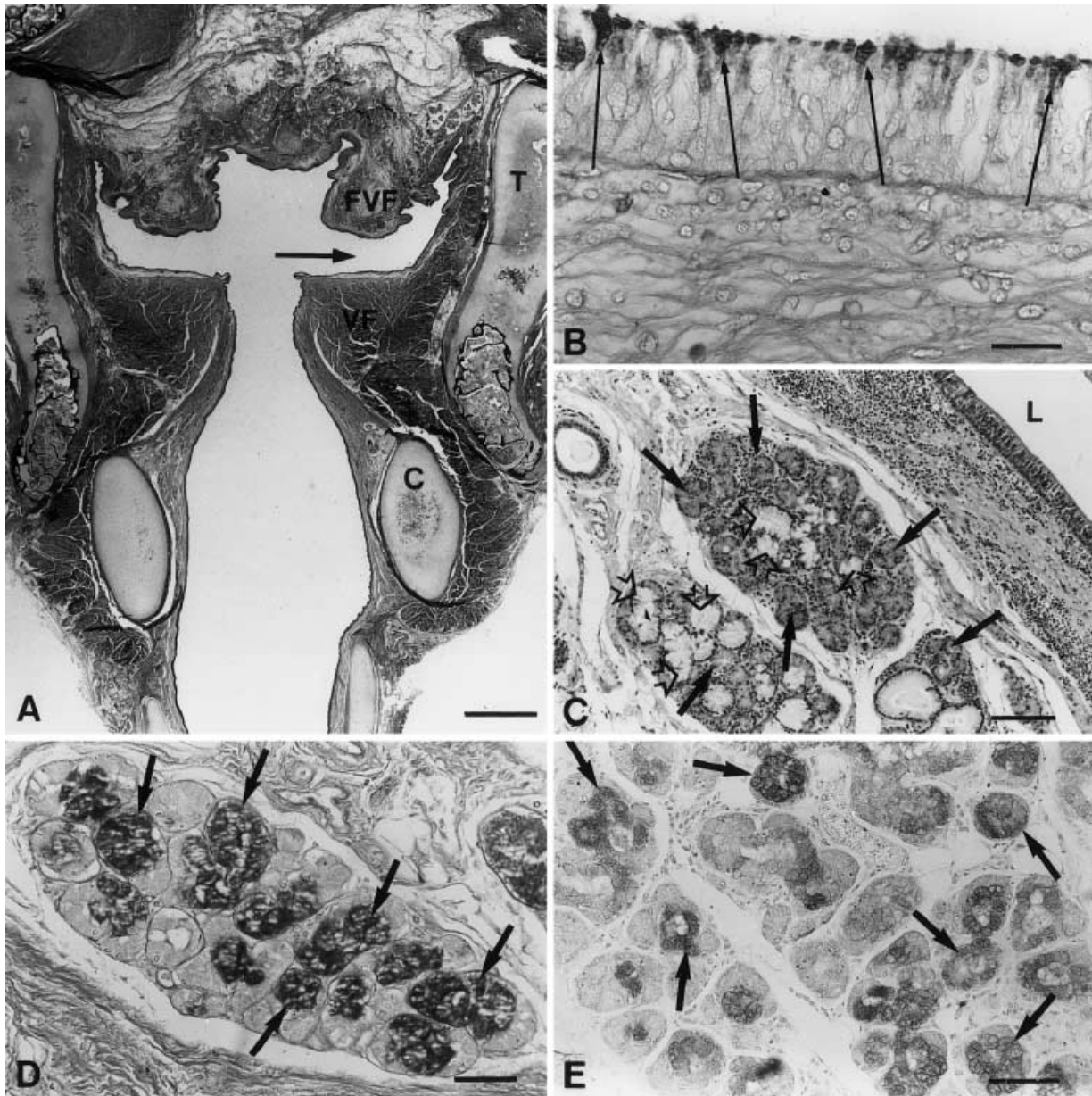


Fig. 1 **A** Frontal section through the larynx of a woman (aged 44 years) at the central part of the vocal folds. Anatomical collection of the University of Kiel. (*T* thyroidal cartilage, *C* cricoid cartilage, *VF* true vocal fold, *FVF* false vocal fold, *arrow* entrance into the ventricle of Morgagni.) Azan-staining. Bar 5,000 μm . **B** Horizontal section through the lining epithelium of the vestibular fold. The secretory product of the goblet cells (*arrows*) reacts positive in the acian-blue staining (pH1) and forms a film on the epithelial surface. Bar 27.5 μm . **C** Frontal section through the vestibular fold demonstrating subepithelial seromucous glands. *Closed black arrows* mark serous parts, *open arrows* mark mucous parts of the lobules. The subepithelial layer is infiltrated by a high number of lymphocytes, (*L* laryngeal lumen). Goldner-staining. Bar 161 μm . **D**, **E** Mucous parts of subepithelial glands inside the vestibular folds react positive (*arrows*) in acian-blue staining, pH1, (**D**, Bar 48 μm) and mPAS staining (**E**, Bar 59 μm)

nized lymphoid tissue at the laryngeal side of the epiglottis and termed the tissue larynx-associated lymphoid tissue (LALT) based on its comparability to gut-associated lymphoid tissue (GALT). The authors also observed the frequent occurrence of lymphoid follicles at the lateral wall of the false vocal folds. The aim of this study is to investigate the false vocal folds in relation to specific physiologic and immunologic characteristics of the lining epithelium and subepithelial structures that are able to prevent laryngeal infection. A morphologic and biochemical analysis of cells and cell products was carried out in order to evaluate mechanisms of immune response, protection of the epithelia and functional consequences for the true vocal folds.

Table 1 Origin and carbohydrate specificities of lectin conjugates used in this study (*BSM* bovine submandibular gland mucin, *Fuc* fucose, *Gal* galactose, *GalNAc* *N*-acetyl-galactosamine, *Glc* glucose, *GlcNAc* *N*-acetyl-glucosamine, *Man* mannose, *Neu5Ac* *N*-acetyl-neuraminic acid)

Lectin	Abbreviation	Carbohydrate specificities	Inhibitor
<i>Bauhinia purpurea alba</i> agglutinin	BPA	Gal- β (1-3)GalNAc, α -GalNAc	Lactose
Concanavalin A agglutinin	ConA	α -Man	α -methyl-Man + α -methyl Glc
<i>Dolichos biflorus</i> agglutinin	DBA	α -GalNAc	GalNAc
<i>Griffonia simplicifolia</i> I agglutinin	GSA-I	α -GalNAc> α -Gal	GalNAc+Gal
<i>Griffonia simplicifolia</i> II agglutinin	GSA-II	α (β)-GlcNAc	GlcNAc
<i>Limulus polyphemus</i> agglutinin	LPA	Neu5Ac α (2-3)GalNAc>Neu5Ac α (2-6)GalNAc	BSM
<i>Maackia amurensis</i> agglutinin	MAA	Neu5Ac α (2-3)Gal	Neu5Ac
<i>Arachis hypogaea</i> agglutinin	PNA	D-Gal- β 1-3)GalNAc	Gal
<i>Sambucus nigra</i> agglutinin	SNA	Neu5Ac α (2-6)Gal	Neu5Ac
Glycine max agglutinin	SBA	α + β GalNAc	GalNAc
<i>Ulex europaeus</i> I agglutinin	UEA-I	α -L-Fuc	Fuc
Wheat germ agglutinin	WGA	α -GlcNAc> α -GalNAc>Neu5Ac	GlcNAc, Neu5Ac
Succinylated wheat germ agglutinin	sucWGA	α -GlcNAc> α -GalNAc	GlcNAc

Materials and methods

False vocal folds obtained from 34 human larynges were prepared (20 female, 14 male; aged 49–83 years) from cadavers donated to the Department of Anatomy, Christian Albrecht University of Kiel, Germany. Previous to dissection, the history of each cadaver was studied and laryngeal problems, any common colds during the last weeks of life or other diseases that may have affected laryngeal function were excluded. Thus the larynges analyzed in the present study were free of recent trauma, laryngeal, pharyngeal or esophageal infections or diseases potentially involving or affecting laryngeal function.

Light microscopy

For light microscopy all larynges were fixed in 4% formalin. After 1 week of fixation, the right vestibular fold of each larynx was dissected, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Sections (7- μ m) in three planes were stained with toluidine-blue (pH 8.5), azan, resorcin-fuchsin-thiacine-picric acid, alcian-blue (pH1) and using the Goldner method as well as mild-periodate-Schiff-base (mPAS) staining. The slides were examined with a Zeiss-Axiophot microscope.

Lectin staining

To analyze possible differences in glycosylation of secretion products, the specimens were separated into two different groups, namely women and men.

For lectin-binding experiments, 7- μ m-thick tissue sections were deparaffinized in xylene, rehydrated with a graded series of ethanol, passed into aqua bidest. and finally into TRIS-buffered saline (0.05 M TRIS, 0.15 M sodium chloride, pH 7.4, TBS), supplemented with 0.02 M CaCl₂. Incubation of the sections with various fluorescein isothiocyanate (FITC)-conjugated lectin solutions (10 μ g/ml) was performed in a dark moist chamber at room temperature for 30 min. After washing the slides three times with TBS, sections were mounted in 10% glycerol in TBS or in Vectashield (Vector Laboratories, Burlingame, Calif., USA). Specificity of the lectin-binding was checked in control experiments by mixing the lectin solution with the corresponding inhibiting sugar (see Table 1) at concentrations of 0.2 to 0.5 M prior to incubation for 30 min at room temperature. The origin and the specificities of the lectin conjugates are shown in Table 1. To estimate the autofluorescence of tissue, TBS-mounted sections were used. Slides were examined using a Zeiss-Axiophot microscope equipped for epifluorescence.

Immunohistochemistry

Antimicrobial peptides and IgA

Immunohistochemical staining was done with antibodies against lysozyme (1:200 in TBS, 60 min; A0099; Dako, Glostrup, Denmark), lactoferrin (1:150 in TBS, 60 min; A0186, Dako), human neutrophil defensins (HNP-1-3, 1:800 in TBS, 60 min, T-1034 mouse monoclonal DEF-3, Bachem, Heidelberg, Germany), human beta defensin-1 (HBD-1), human beta defensin-2 (HBD-2) (both 1:500 in TBS, 60 min, sc-10849 and sc-10854 goat polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and IgA (Dako, F0316, 1:20). They were applied using a standard peroxidase-labeled streptavidin-biotin technique, either with microwave heating pretreatment or using conventional methods with trypsinization where appropriate. After counterstaining with hemalaun, the sections were finally mounted with Aquatex (Boehringer, Mannheim, Germany). Only immunostaining of IgA was done in addition to this procedure with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG as the secondary antibody. Three negative control sections were used in each case in which either the primary antibody, the secondary antibody, or the streptavidin-biotin complex was omitted. Sections of human submandibular gland (lysozyme, lactoferrin), human spleen (DEF-3, IgA), and human skin (HBD-1) were used for positive control. All slides were examined by microscope (Zeiss-Axiophot). For HBD-2, normal human skin was used as an additional negative control, since a positive control was not available.

Results

Light microscopy

The ventricular folds were covered with a pseudostratified epithelium (Fig. 1B). The layers consisted of a basal cell layer and superficial columnar layer. Goblet cells were integrated in the epithelium as solitary cells (Fig. 1B) or at some locations as several cell groups forming small intraepithelial mucous glands. The subepithelially located glands of the false vocal cords were of the tubuloacinous, mixed mucous variety and were surrounded by myoepithelial cells (Fig. 1C). The secretory product of the goblet cells and mucous parts of seromucous glands showed a strong positive reaction with mPAS (pH 1; Fig. 1B,D) and with alcian-blue (pH1;

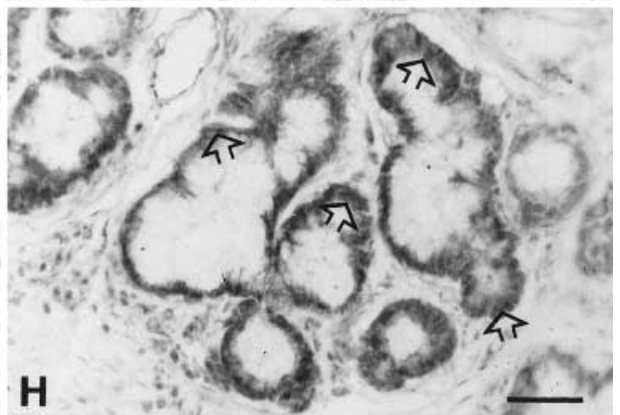
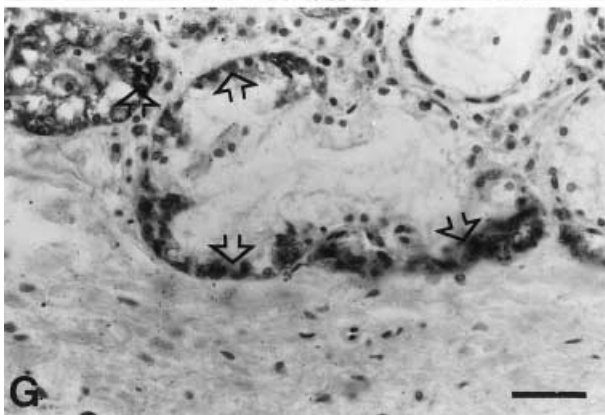
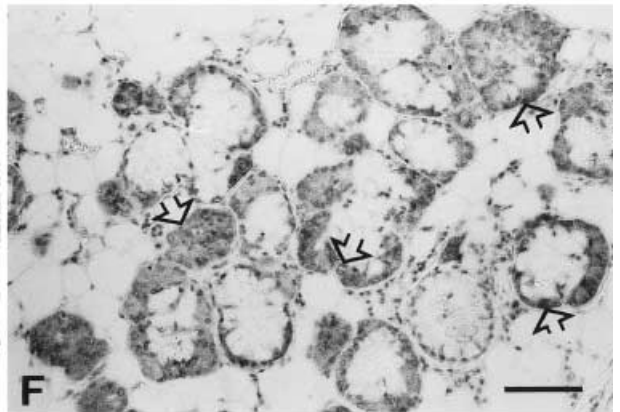
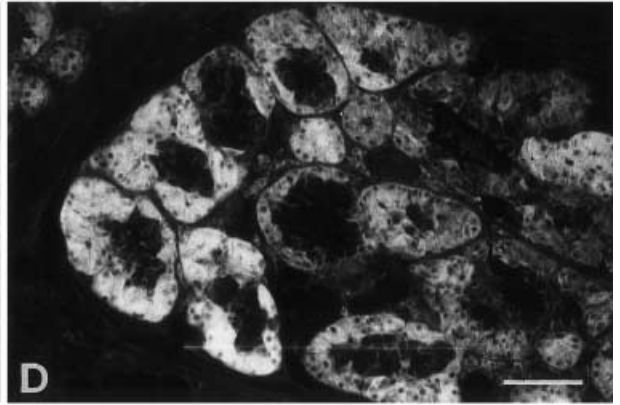
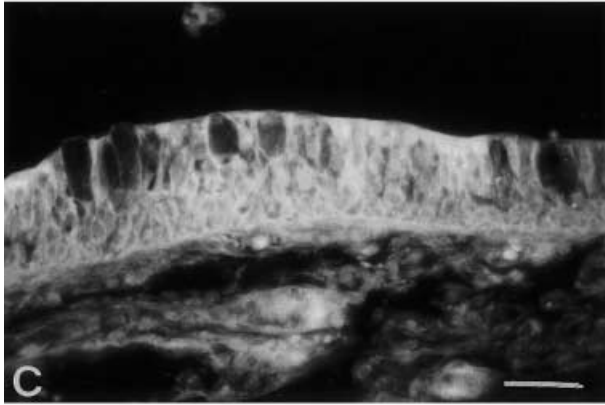
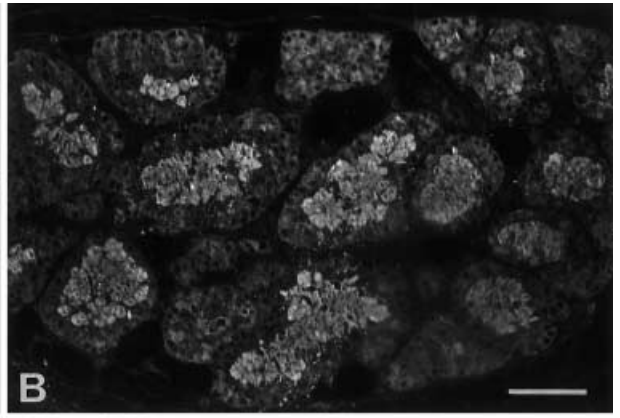
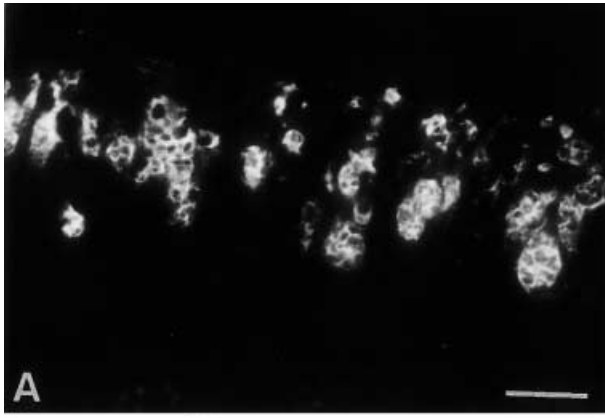


Table 2 Summary of lectin-binding in vestibular fold epithelium and subepithelial seromucous glands [*GC* goblet cell, *EC* epithelial cell, (+) non specific]

Lectin	Men				Women			
	Glands		Epithelium		Glands		Epithelium	
	Serous	Mucous	GC	EC	Serous	Mucous	GC	EC
BPA	-	-	-	-	-	-	-	-
Con-A	+	-	-	+	+	-	-	+
DBA	-	+	+	-	-	+	+	-
GSA I	-	(+)	(+)	-	-	(+)	(+)	-
GSA II	-	-	-	-	-	-	-	-
LPA	-	-	-	-	-	-	-	-
MAA	-	+	+	-	-	+	+	-
MPA	-	+	+	-	-	+	+	-
PNA	-	-	-	-	-	-	-	-
SNA	+	+	+	+	+	+	+	+
SBA	-	-	-	-	-	-	-	-
UEA I	-	+	+	-	-	+	+	-
WGA	+	+	+	+	+	+	+	+
sucWGA	-	+	+	-	-	+	+	-

Fig. 1E). Beneath the epithelium, the lamina propria consisted of loose connective tissue and contained many lymphocytes (Fig. 1C), sometimes arranged in follicles with distinct germinal centers.

Lectin staining

Application of carbohydrate-specific lectins (BPA, Con A, DBA, GSA I, GSA II, LPA, MAA, PNA, SNA, SBA, UEA I, WGA, sucWGA) to deparaffinized tissue sections of healthy vestibular folds resulted in different staining patterns for goblet cells and epithelial cells within the epithelium and the seromucous glands inside the lamina propria of the vestibular folds (Table 2). Binding sites for DBA, MAA, MPA, SNA, UEA I, and sucWGA were uniformly distributed throughout goblet cells and mucous parts of the seromucous glands (Fig. 2A,B), whereas epithelial cells and serous parts of the seromucous glands were negative for DBA, MAA, MPA, SNA, UEA I, and sucWGA. By contrast, Con-A-

staining was restricted to epithelial cells as well as serous parts of seromucous glands and did not show binding sites in goblet cells and mucous parts of seromucous glands (Fig. 2C,D). SNA and WGA-binding was detected in both goblet and epithelial cells as well as serous and mucous parts of seromucous glands. No specific staining was obtained with GSA I. No staining was seen with GSA II, BPA, LPA, PNA, and SBA. No differences were observed in secretions of glycoconjugates between tissue from men and women (Table 2).

Antimicrobial peptide immunostaining

Lysozyme and lactoferrin were found to be present in both the epithelium and in the seromucous glands of the false vocal folds. Lysozyme was produced by the epithelial cells, rendered visible as a red staining of the entire cytoplasm (Fig. 2E,F). Lactoferrin occurred in the epithelial cells as a fine granulation throughout the upper part of the cell. In seromucous glands, both antibodies stained the serous cells (Fig. 2G). Immunohistochemistry with DEF 3 (directed against α defensins 1, 2, and 3) showed positive staining of scattered subepithelial and intraepithelial neutrophils (Fig. 3B), which were also present between serous cells of subepithelial seromucous glands and between the glandular connective tissue (Fig. 3A). Immunoreactivity of HBD-1 was only visible in some of the samples. When present, it occurred as a fine granulation throughout the cytoplasm of epithelial cells or within the serous parts of seromucous glands (Fig. 2H). None of the samples showed immunoreactivity for HBD-2.

IgA immunostaining

Strong immunoreactivity for IgA was observed in the plasma cells of the lamina propria beneath the epithelium

- ◀ **Fig. 2** **A** Histochemical staining with the lectin UEA I. UEA I-binding is uniformly distributed throughout goblet cells, whereas epithelial cells are completely UEA I negative. *Bar* 27.5 μ m. **B** Histochemical staining with the lectin DBA. DBA staining is restricted to the mucous parts of the seromucous glands and does not stain serous parts. *Bar* 27.5 μ m. **C** Histochemical staining with the lectin Con-A. Con-A staining is restricted to epithelial cells and does not stain goblet cells. *Bar* 27.5 μ m. **D** Histochemical staining with the lectin Con-A. Con-A-binding is uniformly distributed throughout serous parts of the seromucous glands, whereas mucous parts are completely Con-A negative. *Bar* 27.5 μ m. **E, F** Immunohistochemical proof of lysozyme (*arrows*) in the epithelium (**E**, *Bar* 27.5 μ m) and serous parts of subepithelial seromucous glands (**F**, *Bar* 74 μ m). **G** Immunohistochemical proof of lactoferrin (*arrows*) in the serous parts of subepithelial seromucous glands. *Bar* 20 μ m. **H** Immunohistochemical proof of human beta defensin 1 (*arrows*) in the serous parts of subepithelial seromucous glands. *Bar* 27.5 μ m

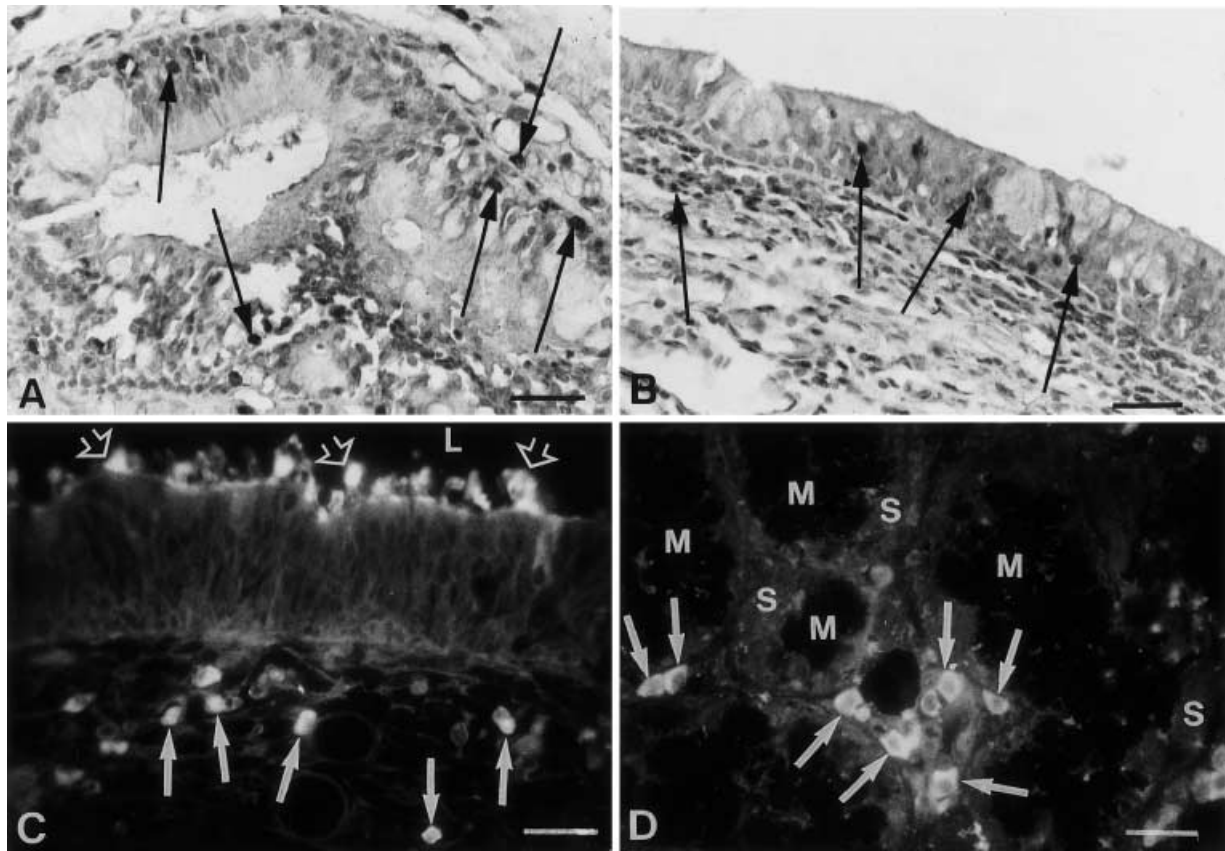


Fig. 3A, B Immunohistochemical proof of human alpha defensins 1, 2, and 3 that stain positive in neutrophils (*arrows*) in serous parts of subepithelial seromucous glands (**A**) and the epithelium (**B**). (**A** Bar 27.5 μm ; **B** Bar 27.5 μm). **C** Immunohistochemical evidence of IgA in the vestibular fold. IgA positive plasma cells (*arrows*) are located subepithelially. The epithelial cells show weak positivity for IgA, which is also contained in the mucous layer (*open arrows*) overlying the epithelium. (*L* laryngeal lumen). Bar 27.5 μm . **D** Immunohistochemical proof of IgA in a seromucous gland of the vestibular fold. IgA-positive plasma cells (*arrows*) are located in the connective tissue between serous (*S*) and mucous (*M*) parts of the gland. Bar 27.5 μm

(Fig. 3C) and in the plasma cells of the connective tissue between seromucous glands (Fig. 3D) as well as in the secretory products of the mucous membrane and the seromucous glands forming a layer on the lining epithelium (Fig. 3C). A somewhat weaker immunoreactivity for IgA was visible inside the epithelial cells and in the serous cells of the seromucous glands.

Discussion

It appears that lysozyme and lactoferrin are actually produced by the mucosal surface of the vestibular folds as well as the serous cells of seromucous glands. Lysozyme and lactoferrin are well known as constituents of airway surface liquid (Singh et al. 2000). Lysozyme is a low-molecular-weight protein showing bacteriostatic and bactericidal activity. It is especially effective in cytolysis

of gram-positive organisms. Moreover, it enhances the antibacterial action of complement and T cells against gram-negative bacteria (Wilhelmus 1985). Lactoferrin is an iron-binding protein that reduces the amount of free iron available for bacteria. It provides both bacteriostatic and bactericidal protection (Oram and Reiter 1979) and plays a role in the primary antibody response, lymphocyte proliferation, cytokine production, natural killer (NK) cell activity and the regulation of complement activation (Kijlstra 1990).

This epithelial antimicrobial defense is supported by neutrophils present in large amounts inside the epithelium and the subepithelial connective tissue of the vestibular folds (Fig. 3A,B). The neutrophils are positive for alpha defensins 1, 2, and 3 (visualized in our study by the antibody DEF3) that have been shown to be amply present in these cells (Ganz et al. 1985; Ganz and Lehrer 1995).

Apart from alpha defensins, mammals produce a second family of defensins, which due to their structural similarity to alpha defensins are termed the beta defensin family. Beta defensins, which occur as ~4-kDa peptides containing 38–42 amino acids, are highly cationic, variably arginine-rich and are distributed in a greater variety of epithelia than alpha defensins (Lehrer et al. 1991; Diamond and Bevins 1998). The two beta defensins analyzed in the present study, HBD-1 and HBD-2, are thought to exert their antimicrobial activity by interacting with membranes of metabolically active bacteria, perhaps by forming pores and causing membrane disruption (Ganz et al. 1985; Lehrer et al. 1989; Hill et al.

1991; Kagan et al. 1994). Other possible roles could include promotion of vestibular fold epithelial healing (Murphy et al. 1993), monocytic (Territo et al. 1989), dendritic and T-cell chemotaxis (Yang et al. 1999; Chertov et al. 2000; Yang et al. 2000), synergistic activity with lysozyme and lactoferrin (Bals et al. 1998), and complement activation (Prohaszka et al. 1997; Prohaszka and Fust 1998). In contrast to other mucosal sites (Bals et al. 1998; Singh et al. 1998; Zucht et al. 1998), our results reveal that HBD-1 is not produced in all vestibular folds analyzed by immunohistochemistry, perhaps because its concentration is below the detection limit of our antibody. Another explanation could be – as shown in a recent study – that early infections can reduce or turn off expression of antimicrobial peptides (Islam et al. 2001), suggesting that its production depends on the status of local bacterial microflora. Having investigated cadavers, we cannot exclude early infection definitively.

HBD-2, one of the only three known human inducible defensins, which is upregulated by contact with gram-negative and gram-positive bacteria as well as *Candida albicans* (Harder et al. 1997) or generated in response to inflammatory cytokines during infection (Haynes et al. 2000), is not detected in our specimens, suggesting that they were free of pathogenic microorganisms that could have induced HBD-2 production or that the peptide is generally not present in the false vocal folds.

Large numbers of goblet cells are present in the epithelium of the vestibular folds. Our lectin-binding experiments reveal that the secretory product of these cells contains carbohydrates including fucose, galactose, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine, and sialic acids. Sialic acids are present in alpha (2-6)-linkage in both goblet and epithelial cells, whereas alpha (2-3)-linkages are detectable only in mucous cells. Mannose was only detectable in epithelial cells and serous parts of seromucous glands. These results were independent of age and sex in the specimens investigated. Our histochemical findings agree with the analysis of Pastor et al. (Pastor et al. 1994). These authors compared the submucosal glands of the epiglottis, the ventricle, and the subglottic region of the larynx with submucosal bronchial glands.

It is known from the gastrointestinal tract that mucus serves several functions. Besides lubrication of the mucosa and water-proofing to regulate epithelial cell hydration, mucins protect mucosal surfaces against potentially harmful substances (Reuter et al. 1992; Irimura et al. 1999). However, a variety of oral and intestinal bacteria have been shown to produce sialidase, an enzyme that can degrade mucins by removing sialic acid (Corfield 1992). In addition, oral and intestinal bacteria synthesize an array of other glycosidases that can attack the oligosaccharide residues of mucins (Schauer 1997).

Recently, it has been shown that bacterial receptors are involved in signal transduction at the molecular level or in mimicking the function of growth factor ligands either coupled to tyrosine kinase activity or to heterotrimeric G protein. The factors lead to a multiplicity of cel-

lular events following their activation, depending on factors such as cellular type, species and/or tissue (Hebert 2000). The bacterial receptors may be surface-bound enzymes with specific binding properties, or sugar-binding proteins on microvilli (Gibbons 1980; Kelm and Schauer 1997). For example, it has been observed that for blocking rotavirus infection of MA104 cells galactose-specific lectins were the most inhibitory (Jolly et al. 2000). Moreover, in vitro experiments give evidence that interaction of *Sambucus nigra* (SNA) with human neutrophils is accompanied by dose-dependent release of lysozyme (Gorudko and Timoshenko 2000). Lectins on macrophages can contribute to host defence by means of mannose receptor (MR) and complement receptor three (CR3), both of which are able to mediate phagocytosis of pathogenic microbes and induce intracellular killing mechanisms (Linehan et al. 2000).

It has also been suggested that IgA supplements this protective activity by incorporation into the mucus layer of mucosal surfaces (Bienenstock 1974). It can interact with functionally diverse cells, including epithelial cells, B and T-lymphocytes, NK cells, cells of the monocyte/macrophage lineage, and neutrophils (Mestecky and McGhee 1987).

All of the latter cell types are present on and in the human false vocal folds and belong to the laryngeal mucosal immune system. Here, B and T-lymphocytes, NK cells, and cells of the monocyte/macrophage lineage often form organized mucosa-associated lymphoid tissue (MALT). Kracke et al. (1997) analyzed the frequency of laryngeal MALT in young children aged 7 to 671 days. As they found the tissue in nearly 80% of the children investigated and termed it larynx-associated lymphoid tissue (LALT). In a further study (Hiller et al. 1998), the authors state, that the clinical relevance of LALT as a physiologic entry site for antigens has been unknown to date in humans and needs to be clarified.

From these findings, it can be assumed that the microbial defense properties of the epithelium of the vestibular folds are of similar significance to those known from the intestinal epithelium. Various lectin-binding sites have been detected in mucin-producing goblet cells and mucous parts of subepithelial seromucous glands. Pilette et al. (2001) showed high levels of IgA inside and on the surface of the epithelium of the respiratory tract. Our data underline this finding for the respiratory epithelium of the larynx and show that IgA is also produced by plasma cells of both seromucous glands and the epithelium of the vestibular folds. IgA could interact with T and B lymphocytes as well as macrophages that are present inside the epithelium and the underlying *substantia propria*, where LALT is present.

Further investigations of the false vocal folds of the larynx will be necessary to evaluate the importance of different substances comprising the protective mucus layer, i.e. antimicrobial peptides, mucins and mucin-associated peptides like trefoil factor peptides (TFF) in healing and disease (Zasloff 2002; Paulsen 2002; Hoffmann et al. 2001). An understanding of the exact

mechanism of production and regulation of these substances at the mucosal surface of the vestibular folds will provide further insight into the occurrence of laryngitis. Factors controlling the production of supralaryngeal mucosa-associated proteins and peptides are widely unknown and it is likely that some infection risk factors like old age, changes in hormonal status (post-menopausal women) or immunodeficiency are associated with downregulation of these substances. Antimicrobial peptides, mucins and TFF peptides may therefore offer a more refined approach to the management of laryngitis.

It can be concluded that the glands and the epithelium of the vestibular folds seem to play an important role in microbial defense, receptor-mediated binding of bacteria and the protective mucus layer (Corfield 1992). This mucus layer is mainly secreted on the true vocal folds and therefore plays not only an important role in lubrication of the true vocal folds and breath moistening, but also in protection of the true vocal folds.

Loss of mucus production and secretion on the true vocal folds could reduce the extent and degree of vocal fold closure during phonation, leading to aperiodicity in the vocal signal and thereby diminishing vocal quality. Such vocal folds are also likely to be infected by bacteria or viruses due to reduction or absence of the protective mucus layer.

Moreover, the role of LALT in immune defense is still unclear. In future the task of LALT in correlation with antimicrobial defense mechanisms in the larynx has to be analyzed more precisely.

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