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The spectrum of cytokeratins expressed in the adult human cornea, limbus and perilimbal conjunctiva

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Summary. The aim of this study was to detect a spectrum of cytokeratins (CK) present in the adult human cornea, limbus and perilimbal conjunctiva.

Cryosections from seven corneo-scleral discs were fixed, and indirect immunofluorescent staining was performed using antibodies directed against CK1-CK10 and CK13-CK20. The percentage of positive cells was calculated in the epithelium of the cornea, limbus and perilimbal conjunctiva. Quantitative real time RT-PCR (qRT-PCR) was used to detect CK6 and CK18 expression in the corneal and conjunctival epithelium.

The most intense staining present throughout the cornea was observed for CK3, CK5 and CK14; CK19 was found at the corneal periphery only. CK4 and CK10/13 revealed mild to moderate positivity mostly in the superficial layers of the cornea. The suprabasal cell layers of all examined areas showed a strong positivity for CK16. A heterogeneous staining pattern with a centrifugal decrease in the signal was observed for CK8 and CK18. CK5/6, CK14 and CK19 were present in the limbus, where a positive signal for CK3 was observed in the suprabasal and superficial cells only. In contrast to the cornea, CK15 appeared in the basal and suprabasal layers of the limbus. The perilimbal conjunctiva showed strong immunostaining for CK10/13, CK14 and CK19. A moderate signal for CK7 was detected in the superficial layers of the conjunctiva. qRT-PCR confirmed CK6 and CK18 expression in the corneal and conjunctival epithelium.

The detailed characterization of the corneal, limbal and perilimbal conjunctival epithelium under normal circumstances may be useful for characterizing the changes occurring under pathological conditions.

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Introduction

The cornea forms the outermost surface of the human eye, transmits light into the inner eye and has a refractive power of approximately 43 dioptres (Olsen, 1986). The cornea's outer surface is bordered by epithelium and its basal membrane, while its inner surface is bordered by the corneal endothelium with the underlying Descemet's membrane. The widest central part of the cornea is composed of the stroma (extracellular matrix with scattered keratocytes). The peripheral cornea continues through a transition zone - the limbus (border of the cornea and sclera) - to the conjunctiva and sclera. The conjunctiva consists of stratified non-keratinizing epithelium and varies in thickness and appearance from the eyelid margin to the limbus (Nelson and Cameron, 2005).

The approximately 50 μm thick corneal epithelium is composed of nonkeratinized, stratified cells. It consists of five to six layers: a monolayer of columnar basal cells, two or three layers of wing cells and two or three layers of superficial cells. The differentiation process requires about 7 to 14 days, and then the superficial cells are desquamated into the tear film (Hanna et al., 1961). The corneal epithelium is renewed throughout life from basal cells and from a population of limbal epithelial stem cells, which proliferate and migrate centripetally to the central epithelium (Davanger and Evenson, 1971; Thoft and Friend, 1983; Tseng, 1989; Chang et al., 2008). The epithelium of the eye contributes to the maintenance of the optically smooth eye surface, provides a barrier to external biological and chemical insults and protects the ocular surface from

microbial attack (Sack et al., 2001).

Cytokeratins (CKs) are epithelial-specific intermediate filaments, which are expressed in a tissue-specific and differentiation-dependent manner (Moll et al., 1982). CKs can be divided into two subfamilies: acidic type I CKs 9-20 (the genes of which are present on chromosome 17, except the gene for CK18, which is located on chromosome 12) and neutral to basic type II CKs 1-8 (whose genes are located on chromosome 12), (Moll et al., 2008). Each CK is a product of a distinct gene (Upasani et al., 2004). CKs are very stable, relatively resistant to degradation and very antigenic (Morgan et al., 1987). Their function is not only to maintain the shape of a cell and to protect the cell from mechanical stress, but they are also important for differentiation and specialized functions, including protection from apoptosis, stress and injury, the intracellular transduction of signals and malignant transformation (Oshima et al., 1996; Caulin et al., 2000; Zatloukal et al., 2000; Kim et al., 2006). Some CKs, particularly CKs 5, 7, 8/18, 19 and 20, exhibit characteristic expression patterns in human tumors; therefore, they have great importance in the immunohistochemical diagnosis of carcinomas (Moll et al., 2008).

Simple epithelium expresses predominantly CKs 8, 18, 7, 17 and 19 (Leube et al., 1986; Moll et al., 2008); additionally, CKs 7 and 19 are characteristic of glandular epithelium (Ramaekers et al., 1987). The basal cell layer of the stratified epithelium expresses CKs 5 and 14, while the cells of the superficial layers express CKs 1 and 10 or 4 and 13, depending on the state of epithelial keratinization and differentiation (Moll et al., 1982; Morgan et al., 1987). CK6 and CK16 are typical of hyperproliferative epithelium (van der Velden et al., 1999; Franssen et al., 2004). CK15 is a basal keratinocyte keratin and has been proposed as a potential marker of stem cells in the hair follicle bulge (Lloyd et al., 1995; Lyle et al., 1998; Liu et al., 2003) as well as in limbal stem cells (Yoshida et al., 2006).

Mutations in individual cytokeratin genes cause a variety of human autosomal-dominant familial diseases, in some of which ocular pathology has been described. In the hereditary blistering skin disease epidermolysis bullosa simplex, conjunctival and corneal blistering occurs due to various point mutations in the CK5 and CK14 genes (Coulombe et al., 1991; Lane et al., 1992; Lin et al., 1994). In Meesmann's corneal dystrophy, mutations in the CK3 and CK12 genes cause intraepithelial microcyst formation in the corneal epithelium (Irvine et al., 1997). Mutations in the genes for CKs 6, 16 and 17 or CKs 1 and 10 are associated with congenital pachyonychia types I and II or with bullous congenital ichthyosiform erythroderma, respectively, where no known eye pathology has been described (Chipev et al., 1992; Rothnagel et al., 1992; McLean et al., 1994; Bowden et al., 1995; McLean et al., 1995). Similarly, mutations in CK4 and CK13 are associated with white sponge nevus, without eye pathology (Richard et al., 1995; Rugg et al., 1995). No disease-causing mutations in the human CK7, CK15, CK19 or CK20 genes have yet been found (Owens and Lane, 2004; Moll et al., 2008).

The expression of CK3, which forms a dimmer with CK12, is well documented in the corneal epithelium, and both are believed to be cornea-specific (Moll et al., 1982; Lauweryns et al., 1993a). CK4 is expressed in the central cornea, CK13 in the peripheral cornea and CK19 in the peripheral cornea and in the basal cells of the limbus and conjunctiva (Lauweryns et al. 1993a,b). In the corneal epithelium CKs 7, 8, 11, 14, 16, 18 and 19/5 have been previously detected (Ross et al., 1995; Cockerham et al., 2002). In the adult human corneal endothelium, no CKs have been detected with the exception of the CK pair 8/18 (Kasper et al., 1992; Merjava et al., 2009).

The aim of this study was to determine, in detail, the presence of CK1-CK10 and CK13-CK20 in healthy human corneal epithelium, limbus and perilimbal conjunctiva, which can help the characterization of corneas under pathological conditions.

Materials and methods

Specimens

The study followed the tenets set out in the Declaration of Helsinki. Eleven corneo-scleral discs (11-17 mm in diameter, age from 38 to 74, mean age of 55.1±14.1 years) not acceptable for transplantation because of low endothelial density or positive serology results of the donor, obtained from the Ocular Tissue Bank Prague, were used. The time between death and storage in liquid nitrogen did not exceed 24 hours. First, seven corneo-scleral discs were dissected, snap frozen in liquid nitrogen, embedded in Optimal Cutting Temperature Compound and stored at -70°C. Tissues were cryosectioned at a thickness of 7 µm, and four slices were mounted per slide. Finally, four corneal and conjunctival epithelial imprints on Biopore Millicell membranes (MILLICELL®- CM, PICM 01250, Millipore, Bedford MA) were used for the detection of CK6 and CK18 by quantitative real time RT-PCR (qRT-PCR) (total mRNA was isolated from cells on the Millicell membranes). For CKs which are not expressed in the cornea or conjunctiva, epithelia from breast skin (for CK2e), skin from the palm of the hand and the sole of the foot (for CK9, CK10), the mammary gland (for CK17) and the stomach mucosa (for CK20) were used as positive control tissues.

Indirect fluorescent immunohistochemistry

Seven different corneal samples were used to prepare cryosections for indirect immunofluorescence. Three cryosections on each slide were stained with a single antibody. The fourth section was used as a negative control (primary antibody omitted). Two

independent experiments were performed. Sections were fixed with cold acetone for 10 minutes, rinsed in phosphate buffered saline (PBS) and then incubated with the primary antibodies diluted in PBS containing 1 % bovine serum albumin (BSA) for 1 hour at room temperature. The following mouse monoclonal or goat (CK1) antibodies and dilutions were used: anticytokeratin 1 (1:250), anti-cytokeratin 3 (1:50), anti-cytokeratin 5 (1:400), anti-cytokeratin 6 (1:20), anticytokeratin 10 (1:50) (Santa Cruz Biotechnology, Santa Cruz, USA); anticytokeratin 2e (1:10), anticytokeratin 9 (1:15), anticytokeratin 16 (1:30), anticytokeratin 17 (1:25) and anticytokeratin 20 (1:15) (Acris Antibodies GmbH, Hiddenhausen, Germany); anticytokeratin 4 (1:200) (Sigma, St. Louis, USA); anticytokeratin 5/6 (1:40), anticytokeratin 7 (1:50), anticytokeratin 10/13 (1:25), anticytokeratin 18 (1:50) and anticytokeratin 19 (1:50) (DakoCytomation, Glostrup, Denmark); anticytokeratin 8 (1:400), anticytokeratin 14 (1:50) and anticytokeratin 15 (1:100) (Chemicon International Inc., Temecula, USA). The specimens were washed three times with PBS and incubated with the appropriate secondary antibodies (fluorescein isothiocyanateconjugated or rhodamine-conjugated antimouse or antigoat IgG, Jackson ImmunoResearch Laboratories, West Grove, USA) for 1 hour at room temperature. After rinsing in PBS the slices were mounted with Vectashield - propidium iodide or 4,6diamidino2phenylindoldihydrochlorid (DAPI) (Vector Laboratories, Inc. Burlingame, USA) to counterstain the DNA within the nuclei.

Specimen assessment

Slices were examined by fluorescent microscopy using an Olympus BX51 (Olympus Co., Tokyo, Japan) at a magnification of 100-400x. Images were taken using a Vosskühler VDS CCD-1300 camera, (VDS Vosskühler GmbH, Germany), and NIS Elements software (Laboratory Imaging, Czech Republic) was used for cell analysis. The corneal, limbal and perilimbal conjunctival epithelia were evaluated separately. At least 300 epithelial and 100 endothelial cells directly connected to DM were examined, and the percentage of positive cells was calculated. To grade the intensity of cell staining, a scale was used: N: negative, 1: mild, 2: moderate, 3: intense, and 4: very intense staining. The mean range was calculated from three sections and two experiments.

Quantitative real time RT-PCR (qRT-PCR)

Total RNA was isolated from four corneal and conjunctival epithelial imprints on Millicell membranes using Rneasy Plus Microkit (Qiagen, Hilden, Germany). At least two corneal and conjunctival specimens were used per experiment. RNA was reverse transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and random hexamers followed by PCR amplification with Sybr green master mix (Roche Diagnostics, Mannheim, Germany). The following specific oligonucleotides for CK6, CK18 and the housekeeping gene glyceraldehyde-3-phospate

Table 1. Fluorescent immunhistochemistry of different cytokeratins (CK) in individual layers (basal, suprabasal, superficial) of healthy corneal, limbal and perilimbal conjunctival epithelium.

CK type	Cornea			Limbus			Conjunctiva		
	basal	suprabas.	superfic.	basal	suprabas.	superfic.	basal	suprabas.	superfic.
average % of positive cells / intensity									
CK 1	21/1	26/1	26/1	N	10/1	16/1	N	15/1	15/1
CK 2e	N	N	N	N	N	N	N	N	N
CK 3	52/2	66/2	91/3	N	28/2	8/1	N	N	N
CK 4	19/1	46/1	70/2	N	37/1	63/2	N	54/2	77/3
CK 5	51/2	51/2	54/2	24/2	46/2	47/2	28/2	43/2	47/2
CK 6	N	14/1	11/1	N	7/1	9/1	N	N	9/1
CK 5/6	90/2	89/2	86/2	63/1	86/2	86/2	57/1	87/2	84/2
CK 7	N	N	N	N	N	N	N	N	31/2
CK 8	34/1	45/1	69/2	42/1	38/1	56/2	19/1	30/2	56/2
CK 9	N	N	N	N	N	N	N	N	N
CK 10	N	N	N	N	N	N	N	N	N
CK 10/13	10/1	29/1	45/2	N	39/2	62/2	10/1	70/2	82/3
CK 14	70/2	69/2	52/2	87/2	63/2	46/2	83/3	66/2	56/2
CK 15	N	N	N	46/2	21/1	N	67/2	23/1	17/1
CK 16	33/1	78/2	61/2	N	12/1	N	N	10/2	N
CK 17	N	N	N	N	N	N	N	N	N
CK 18	62/1	43/1	42/1	16/1	21/1	32/1	N	N	26/1
CK 19	54/2*	55/1*	65/2*	63/2	59/2	83/3	71/2	59/2	88/3
CK 20	N	N	N	N	N	N	N	N	N

Number of cells stained: % (average percentage) / N: negative, 1: mild, 2: moderate, 3: intense, and 4: very intense staining. *: assessed from the peripheral parts of the corneal epithelium.

dehydrogenase (GAPDH) were used: human CK6, sense primer 5' -agtttgcctccttcatcgac- 3', anti-sense primer 5' -cagcagggtccactttgttt- 3'; human CK18, sense primer 5' -cctgctgtccgtgtccat- 3', anti-sense primer 5' -ggaccggtagttggtgga- 3'; human GAPDH, sense primer 5' -agc cac atc gct cag acac- 3', anti-sense primer 5' -gcc caa tac gac caa atcc- 3'. The reactions were run in triplicate on a LC480 thermocycler (Roche Diagnostics).

The gene expression for CK6 and CK18 was quantified using a relative quantification model with efficiency correction using the following formula: ratio = $(E_{target})^{Ct\ target\ (control\text{-}treated)} / (E_{ref})^{Ct\ ref\ (control\text{-}treated)}$ according to Pfaffl (2001). The efficiency for each gene was estimated by the dilution calibration method. The sample with the lowest cytokeratin expression was taken as the calibrator (expression = 1), and the experiment was done

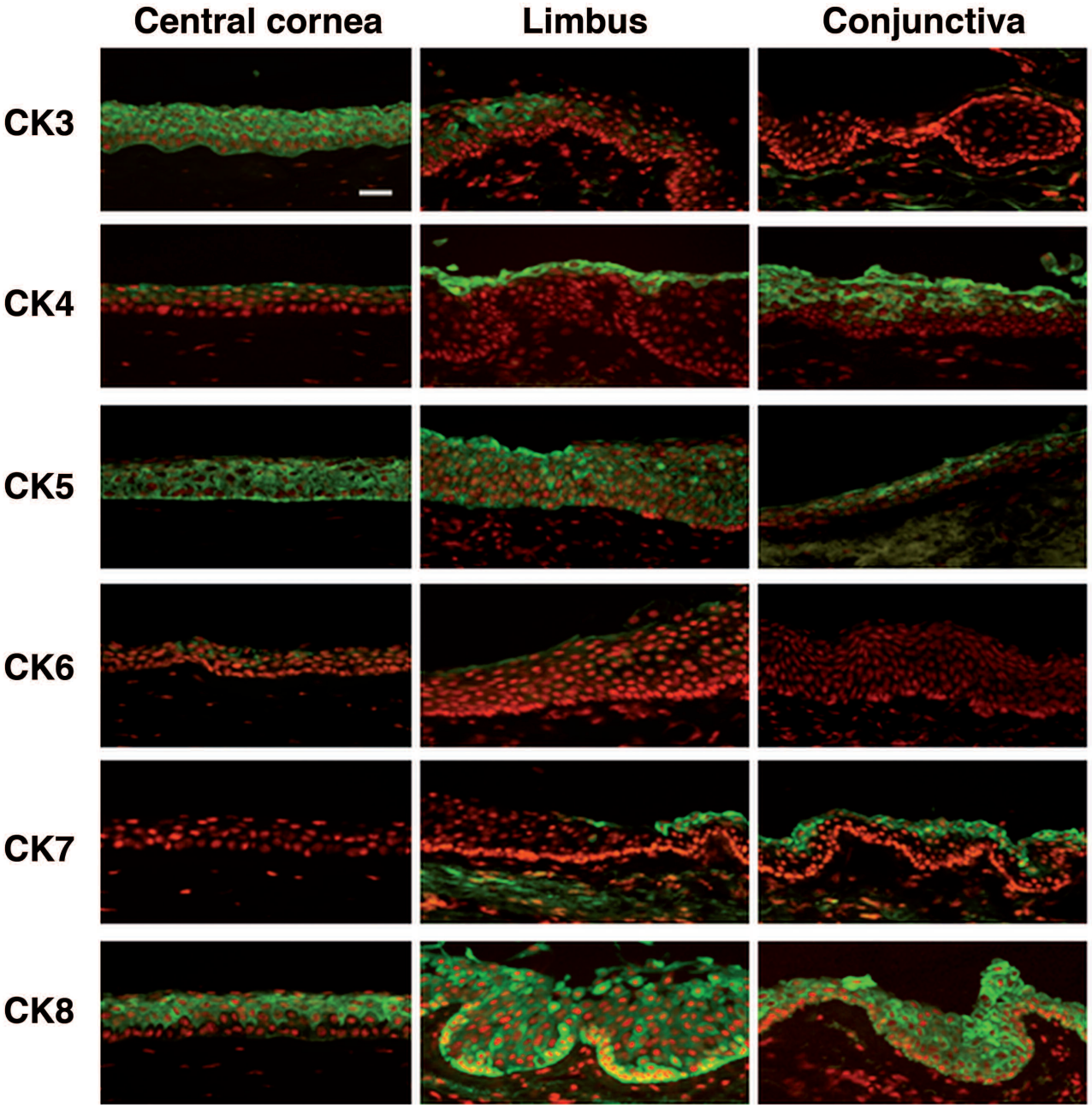

Fig. 1. The presence of neutral to basic cytokeratins (CK1 - CK8) in the epithelium of the adult human central cornea, limbus and conjunctiva. Indirect immunofluorescent staining was used. Scale bar: 50 μm.

in duplicate.

Results

The results of the immunohistochemical analysis of normal tissue stained with antibodies to various CKs are shown in Table 1 and representative figures are presented in Figs. 1, 2.

In the cornea moderate to intense positivity for CK3 was observed in most cells (70%; mean percentage from the values obtained in the basal, suprabasal and superficial layers). CK5, 5/6, 14 and 19 revealed moderate positivity dispersed throughout the corneal epithelium; CK5 was present in 52% of all detected

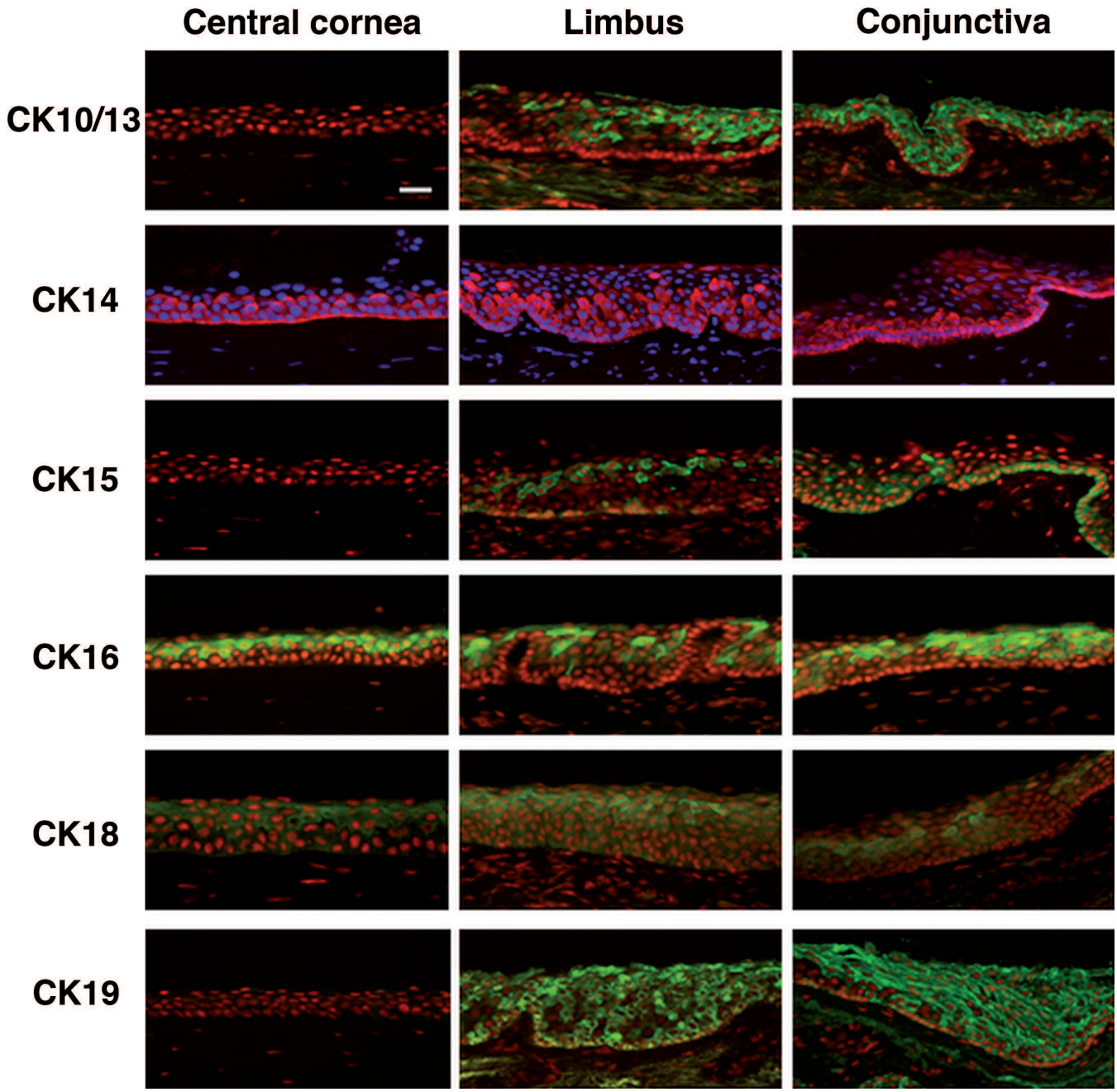

Fig. 2. The presence of acidic cytokeratins (CK9 - CK20) in the epithelium of the adult human central cornea, limbus and conjunctiva. Indirect immunofluorescent staining was used. Scale bar: 50 μm.

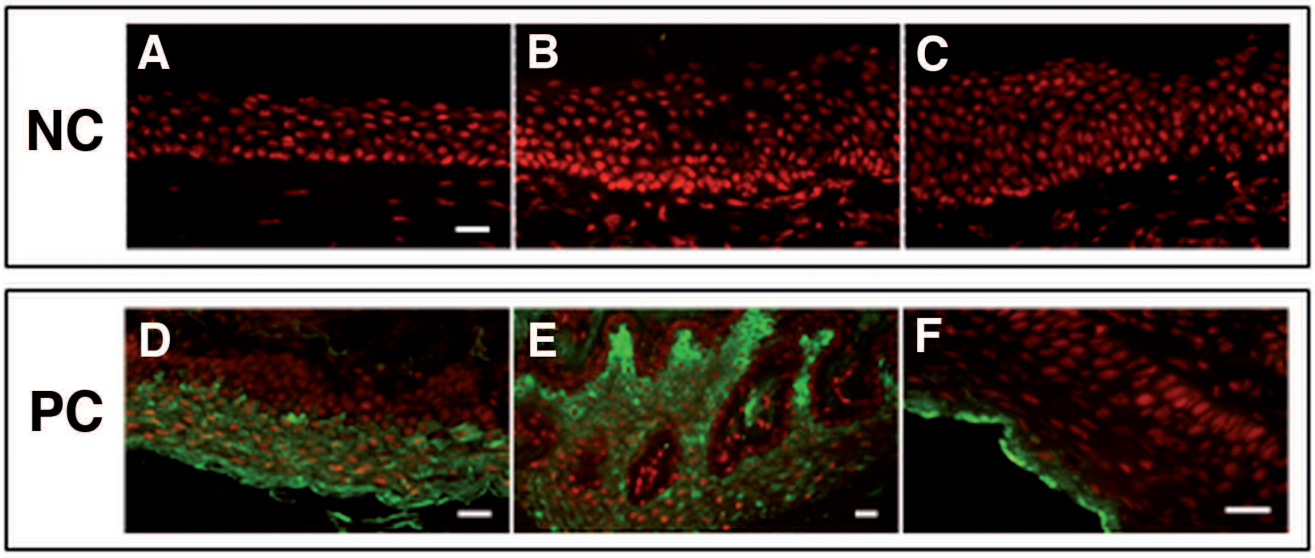

Fig. 3. Negative control (NC) of the corneal **(A),** limbal **(B)** and conjunctival **(C)** epithelium. Epithelia from breast skin for CK2e **(D)**, skin from the palm of the hand and the sole of the foot for CK9 **(E)** and CK10 **(F)** were used as positive control tissues (PC). Scale bar: 50 μm.

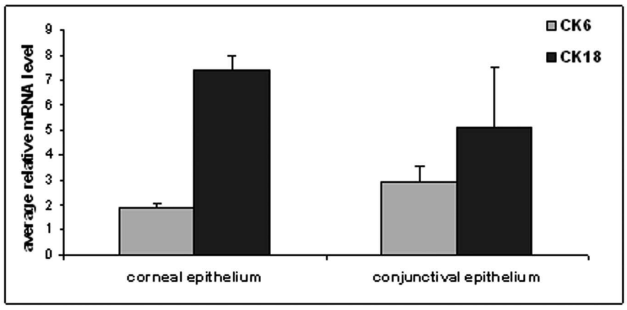

Fig. 4. Average relative expression of mRNA encoding cytokeratin 6 (CK6) and cytokeratin 18 (CK18) in four corneal and conjunctival epithelium samples. Relative CK6 and CK18 expression was normalized to GAPDH expression, and the sample with the lowest cytokeratin expression was taken as the calibrator (expression = 1).

cells; CK5/6 was present in 88% of all detected cells. CK6 revealed only mild positivity in 12% of the suprabasal and superficial cells. CK14 was detected in 70% of cells located in the basal layer and in 52% of cells of the superficial corneal layer. CK19 was present in 58% of cells; however, the immunostaining occurred mostly at the periphery of the cornea, while almost no positivity was detected in the central part of the cornea. A signal for CK16 was observed in all corneal epithelial layers; the most intense staining and the highest percentage of positive cells was present in the suprabasal layer (78% of cells). CK4 and CK10/13 revealed mild to moderate positivity in all corneal layers, where the percentage of positive cells increased from the basal (19 and 10%, respectively) to the superficial layer (70 and 45%, respectively), but the immunostaining for CK10/13 was present only in the peripheral part of the corneal epithelium. CK8 and CK18 were detected throughout all the corneal epithelial layers, with the majority of CK8 positive cells in the superficial layer (69%) and the highest number of CK18 positive cells in the basal layer (62%). All corneal epithelial layers were mildly positive for CK1. CK15 was expressed in a few epithelial basal cells at the corneal periphery in two of seven specimens. No staining for CK2e, CK7, CK9, CK10, CK17 and CK20 was observed in any corneal specimen. No signal was present in any of the negative controls for any of the studied CKs (Fig. 3a, b, c). The staining for CK2e, CK9, CK10, CK17 and CK20 is shown in the appropriate positive controls (Fig. 3d-f).

The strongest positivity in the limbus was detected for CKs 5, 5/6 and 14 (where the staining decreased from the basal to the superficial layers) as well as CK19. Heterogenous staining in each limbal layer was observed for CKs 8 and 18 (mean 45% and 23%, respectively). CK3 was present in 8% of the superficial and 28% of the suprabasal cells, while the basal layer was negative. The immunostaining declined from the border between the cornea and limbus towards the conjunctiva, in which staining for CK3 was negative. A signal in the superficial and suprabasal layers was observed for CK4, CK6, CK10/13 and CK1. CK16 was present in 12% of the suprabasal cells. In contrast to its absence in the cornea, CK15 appeared in the basal and suprabasal cells of the limbus.

The strongest positivity in the perilimbal conjunctiva was detected for CK5, 5/6, 14 and CK19, for which the

staining decreased from the basal to the superficial layers, and for CK10/13, 4 and CK1, for which the staining decreased in the opposite direction. CK16 was present in 10% of the suprabasal cells. Heterogenous staining in each conjunctival layer was observed for CK8 (mean 35% of cells). In contrast to the cornea and limbus, CK18 was detected only in the superficial layer (26% of cells) of the conjunctiva. CK15 appeared in each conjunctival layer, with the strongest positivity seen in the basal cells. Unlike the cornea and limbus, CK3 was absent throughout the whole conjunctival epithelium.

Of all the tested CKs, only CK8 and CK18 were present in the corneal endothelium in 46 and 23% of the cells, respectively. None of the tested CKs was expressed by keratocytes in the stroma.

The expression of CK6 and CK18 was confirmed by qRT-PCR. Relative CK6 and CK18 expression was normalized to an endogenously expressed housekeeping gene (GAPDH). Our data clearly showed the lower expression of CK6 and the higher expression of CK18 in both corneal and conjunctival epithelium (Fig. 4).

Discussion

This study describes in detail the CK spectrum in the human cornea, limbus and perilimbal conjunctiva. We have found that the expression of CK3, a corneal epitheliumspecific protein (Moll et al., 1982), decreases centrifugally from the superficial to the basal corneal layers. Moreover, we have found it in the suprabasal cells and in a minority of the superficial limbal cells, compared to the completely negative basal limbal cells. The absence of CK3 from the basal limbal cells makes it possible not only to distinguish between corneal and limbal cells, as necessary for the diagnosis of limbal stem cell deficiency (Donisi et al., 2003), but also between basal cells and cells from the upper limbal layers. This can be helpful for the subsequent separation and identification of limbal epithelial stem cells.

CK19, as a minor cytoskeletal component of the corneal epithelium, has been described as one of the major components in the conjunctival epithelium (Kasper et al., 1988; Elder et al., 1997; Kivelä and Uusitalo, 1998; Pitz and Mol 2002; Schlotzer-Schrehardt and Kruse, 2005). It exhibits the opposite direction in its labeling gradient than does CK3. The CK19 expression pattern through the central epithelium of the cornea is still a matter of some controversy. Our and several other studies have reported that CK19 is located in all layers of the conjunctival and limbal epithelium and that its presence decreases in the peripheral part of the cornea, and finally disappears in the central corneal epithelium (Kasper et al., 1988; Lauweryns et al., 1993a; Pitz and Mol, 2002). On the other hand, a few studies have found various numbers of CK19-positive cells in the central cornea (Chen et al., 2004; Yoshida et al., 2006). These differing results show that besides the methodological approach used in tissue processing, different antibody sensitivities or different fixation methods may contribute to controversial results (Mygind et al., 1988). On the other hand, we have seen no differences in staining for CK4, 5, 6, 8, 14, 16 or 18 between non-blocked sections and sections blocked in 2.5% BSA. It was postulated that the condition of the tissue used, including the time between death and processing of the tissue, may affect cytokeratin expression (Di Iorio et al., 2005). In our experiments the time between death and storage in liquid nitrogen did not exceed 24 hours.

More pronounced differences between the superficial layer of the cornea and the conjunctiva were found with CK7. This protein was intensely present throughout the surface epithelial layer of the conjunctiva yet absent from the superficial layer of the limbus and cornea. This CK was previously described by Krenzer and Freddo, (1997) only in goblet cells and by Elder et al. (1997) in basal and suprabasal epithelial cells of the central cornea.

The simple epithelial cytokeratins CK8 and CK18 were detected in the central corneal epithelium as well as in the limbal and conjunctival epithelium (confirmed using qRT-PCR). From our broad CK spectrum, only these two cytokeratins were detected in endothelial cells, as was shown previously (Merjava et al., 2009). Lastly, the simple epithelial cytokeratin CK17 was completely negative in our experiments, in contrast to the results of Elder et al., (1997) who found CK17 throughout the whole corneal, limbal and conjunctival epithelium. This could be explained by the different sensitivities of the antibodies used in the two studies.

Stratified epithelia CKs revealed the same expression pattern as was described by other authors (Moll et al., 1982; Morgan et al., 1987; Kurpakus et al., 1990). CK4 was detected predominantly in the superficial cell layers and CK14, as a marker of undifferentiated cells in stratified epithelia, mostly in the basal cells of the central, limbal and conjunctival epithelium, respectively. CK15, a minor cytoskeletal component of stratified tissue (Moll et al., 1982), was detected in the basal cells of stratified squamous epithelia as well as in basal cells of the limbus and conjunctiva (Lloyd et al., 1995). Previously, its expression was described in hair follicle bulge cells in the human scalp (Lyle et al., 1998). Our results support the finding that CK15 is predominantly expressed in the basal layers of the conjunctiva and limbus (Yoshida et al., 2006; Figueira et al., 2007; Lyngholm et al., 2008), an area where limbal epithelial stem cells occur, and thus can be considered as a limbal epithelial stem cell marker. Herein we are reporting for the first time the presence of CK6 in the corneal epithelium. Mild positivity was found when a monospecific antibody against CK6 was used, compared to the intense signal obtained using a dual specific antibody against the CK pair 5/6. These results indicate that the positivity observed using the CK5/6 antibody was predominantly CK5 positivity. CK6 and CK16 were detected mainly in the suprabasal cells of the corneal, limbal and conjunctival epithelium.

Although CK6 and CK16 are among the cytokeratins typical of proliferating cells, and we expected their occurrence in the basal layer of the epithelium, their presence in suprabasal cells is not unique (Sun et al., 1984; Mansbridge and Knapp, 1987; van der Velden et al., 1999). The expression of CK6 in the corneal and conjunctival epithelium was confirmed using qRT-PCR.

From CK1 and CK10, which are typical of keratinizing stratified epithelia (Moll et al., 1982), only CK1 was detected in a low amount, predominantly in the superficial cell layers of the corneal, limbal and conjunctival epithelium, which confirms that corneal epithelial cells are primarily nonkeratinized, but they do differentiate and desquamate (Hanna et al., 1961). Using an antibody against CK10 only, surprisingly no positivity was detected, in contrast to the positivity obtained using an antibody which detected CK10 and CK13 together. It seems likely that this positivity was the result of CK13 staining only, in agreement with the nonkeratinized pattern of the corneal epithelium, because CK13, together with CKs 4, 5 and 14, is typical of nonkeratinising stratified epithelia such as the buccal mucosa and alveolar mucosa (Clausen et al., 1986; Sawaf et al., 1991).

Taken together, our results show that the corneal, limbal and conjunctival epithelium express a wide spectrum of cytokeratins and that the corneal epithelium can be characterized as primary nonkeratinizing stratified epithelium (no CK10 and weak CK1 positivity, strong CK3, 4, 5, 13 and 14 positivity), but with the expression of some simple epithelial CKs (CKs 8 and 18).

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