Virchows Archiv A Pathol Anat (1990) 417:419–426

Virchows Archiv A
Pathological Anatomy and Histopathology

Topography-related expression of individual cytokeratins in normal and pathological (non-neoplastic and neoplastic) human oral mucosa

Marcella Cintorino[1], Roberto Petracca[2], Carla Vindigni[1], Sergio Antonio Tripodi[1], and Paolo Leoncini[2]

[1] Department of Pathology, University of Siena, I-53100 Siena, Italy
[2] Sclavo Research Center, Via Fiorentina 1, I-53100 Siena, Italy

Received March 28, 1990 / Received after revision May 28, 1990 / Accepted May 29, 1990

Summary. Recently, regional changes of cytokeratin patterns in human normal non-keratinized or keratinized oral mucosa have been demonstrated and the expression of individual cytokeratin polypeptides in lesions of oral mucosa has been compared with that of normal tissues. In particular, the presence of cytokeratin 19 in the suprabasal cell layers of oral epithelia has been shown to be strongly correlated with premalignancy. In the present study, we describe the results of an immunohistochemical investigation performed using a monoclonal antibody specific for cytokeratin 1 on normal oral mucosa and benign or malignant oral lesions. We show the different distribution of this polypeptide in non-neoplastic lesions from different sites of oral mucosa and describe the presence of cytokeratin 19. Our results are in agreement with the data obtained previously. In the malignant cases we demonstrate that the distribution of the two cytokeratins is characterized by complementary patterns.

Key words: Cytokeratins – Oral leukoplakia – Oral carcinoma

Introduction

Cytokeratins represent a multigene family of intermediate filament proteins which are expressed in epithelial cells and tissues (Franke et al. 1981; Lazarides 1982; Moll et al. 1982). Biochemical, immunological and molecular biology studies (Fuchs et al. 1981; Eichner et al. 1984; Steinert et al. 1985) indicate that these polypeptides can be divided into two subfamilies: acidic cytokeratins (type I) and basic cytokeratins (type II).

Normally each acidic cytokeratin is co-expressed with a basic one which is specific, thus forming a "keratin pair". Individual polypeptides, as well as pairs of polypeptides, are specific for different epithelial types and can be used as markers of the differentiation programmes of epithelia (Quinlan et al. 1985; Sun et al. 1985; Fuchs et al. 1987). From this point of view, the study of the epithelium lining the oral cavity, composed of keratinizing and non-keratinizing regions, has been important in describing cytokeratin expression in stratified squamous epithelia. Several biochemical and immunohistochemical investigations have shown regional changes in the cytokeratin pattern, (Ouhayoun et al. 1985; Reibel et al. 1985; Clausen et al. 1986; Morgan et al. 1987a, b; Ermich et al. 1988; Bosch et al. 1989). Furthermore, it has been demonstrated that the dorsal surface of the tongue can be divided into at least three compartments that undergo "oesophageal", "skin" and "hair"-type differentiation (Dhouailly et al. 1989).

In pathological modifications of oral epithelium many papers describe immunohistochemical patterns obtained employing poly- or monoclonal antibodies specific for cytokeratins (Löning et al. 1980; Nakai and Mori 1986; Vigneswaran et al. 1989). Recent findings indicate that the expression of cytokeratin 19 in the suprabasal cell layers of oral epithelia is strongly correlated with premalignancy (Lindberg and Rheinwald 1989) or with a hyperproliferative state associated with chronic inflammation (Bosch et al. 1989).

In the present study, we report the results of an immunohistochemical investigation performed using a monoclonal antibody specific for cytokeratin 1 (a marker of epidermal differentiation). The analysis has been performed on sections from human normal oral mucosa and from benign or malignant oral lesions. We also employed a monoclonal antibody recognizing cytokeratin 19 and suggest that the expression patterns of both these cytokeratins may help to understand some of the mechanisms associated with the neoplastic transformation and the biological behaviour of tumours.

Offprint requests to: P. Leoncini

Table 1. Summary of investigated pathological cases

Type of mucosa (no. of cases)	Location	Diagnosis				Total
		Leukoplakia	Leukoplakia with mild to severe dysplasia	Squamous papilloma	Squamous cell carcinoma	
I (26)	Buccal mucosa	4	–	–	–	4
	Vestibular mucosa	4	2	–	3	9
	Floor of the mouth	2	–	–	1	3
	Pilastrum	–	1	–	1	2
	Soft palate	1	1	3	–	5
	Uvula	–	–	2	–	2
	Ventral tongue	–	–	–	1	1
II (10)	Gingiva	2	–	1	2	5
	Hard palate	–	1	1	1	3
	Retromolar pad	–	–	–	2	2
III (5)	Dorsal tongue	1	1	2	1	5
Total no. of cases		14	6	9	12	41

Materials and methods

Normal human tissues used for the characterization of monoclonal antibody SK1 were collected from surgery or autopsy. Biopsies of normal oral epithelium, representing both non-keratinizing and keratinizing mucosa, were obtained from healthy donors. Specimens of oral lesions were collected from the files of the Department of Pathology, University of Siena, Italy.

Oral lesions include 41 biopsies obtained from non-neoplastic and neoplastic lesions subdivided according to a topographic criterion. The cases were chosen to represent each type of oral mucosa. We have used the subdivision suggested by Schroeder (1981), which presents three different types: type I, lining mucosa (cheek, buccal and vestibular mucosa, soft palate, floor of the mouth, uvula, pilastrum); type II, masticatory mucosa (gingiva, hard palate, retromolar pad); type III, specialized mucosa (dorsal tongue).

All samples were immediately fixed overnight at room temperature in a 4% formaldehyhde-buffered solution and then embedded in paraffin. Serial sections (4–5 μm) for each case were stained by haematoxylin and eosin for histopathological reassessment. Further sections were employed for immunohistochemical investigation.

A cytoskeletal extract from human foot sole epidermis was used as immunogen for the production of the monoclonal antibody SK1. Immunization of Balb/c mice and cell fusion conditions were as previously described (Cianfriglia et al. 1983). Supernatants collected from hybridoma cultures were screened both by immunohistochemistry and also by immunoblotting, as well be discussed in detail later. Monoclonal A53-B/A2 specific for human cytokeratin 19 (a gift from Dr. M. Kasper, Gorlitz, GDR), was also employed (Kasper et al. 1987).

Immunohistochemical staining was performed using the immunoperoxidase technique with avidin biotin complex method according to the manufacturer's instructions (Vector Labs, Burlingame, Calif., USA). Briefly, the procedure was as follows. Sections were deparaffinized with xylene, followed by absolute ethanol; endogenous peroxidase activity was blocked using 3% (v/v) hydrogen peroxide in H_2O for 5 min and non-specific staining with normal horse serum for 20 min at room temperature. Incubations with monoclonal antibodies were performed for 60 min at room temperature, using hybridoma supernatants. After washes with phosphate-buffered saline (PBS), sections were incubated with biotinylated anti-mouse immunoglobulins, washed again and incubated with avidin-biotin-peroxidase complex. Finally, enzymatic activity was detected with 3-amino-9-ethylcarbazole; in some cases, sections were weakly counterstained with Mayer's haematoxylin. Before immunostaining with A53-B/A2, tissue sections underwent enzymatic treatment as described in the study of Battifora and Kopinski (1986). Negative controls were performed substituting primary antibodies with a monoclonal antibody specific for an antigen which is absent from the tissue under study.

For immunoblotting extracts from human cultured keratinocytes were separated by 7% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Laemmli 1970) and transferred to nitrocellulose by electroblotting (Towbin et al. 1979). Blots were first stained with Ponceau red, then destained with PBS; unreactive sites were blocked by 3% bovine serum albumin in PBS. The nitro-

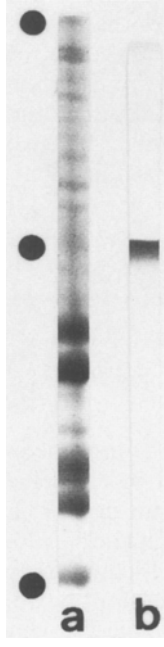

Fig. 1a, b. Immunoblotting using monoclonal antibody SK1: **a** 7% SDS-PAGE of an extract from human cultured keratinocytes (*dots* indicate molecular weight markers 94, 68 and 43 kDa); **b** corresponding immunoblotting showing the specificity of the antibody for a polypeptide (68 kDa), slightly visible on the gel

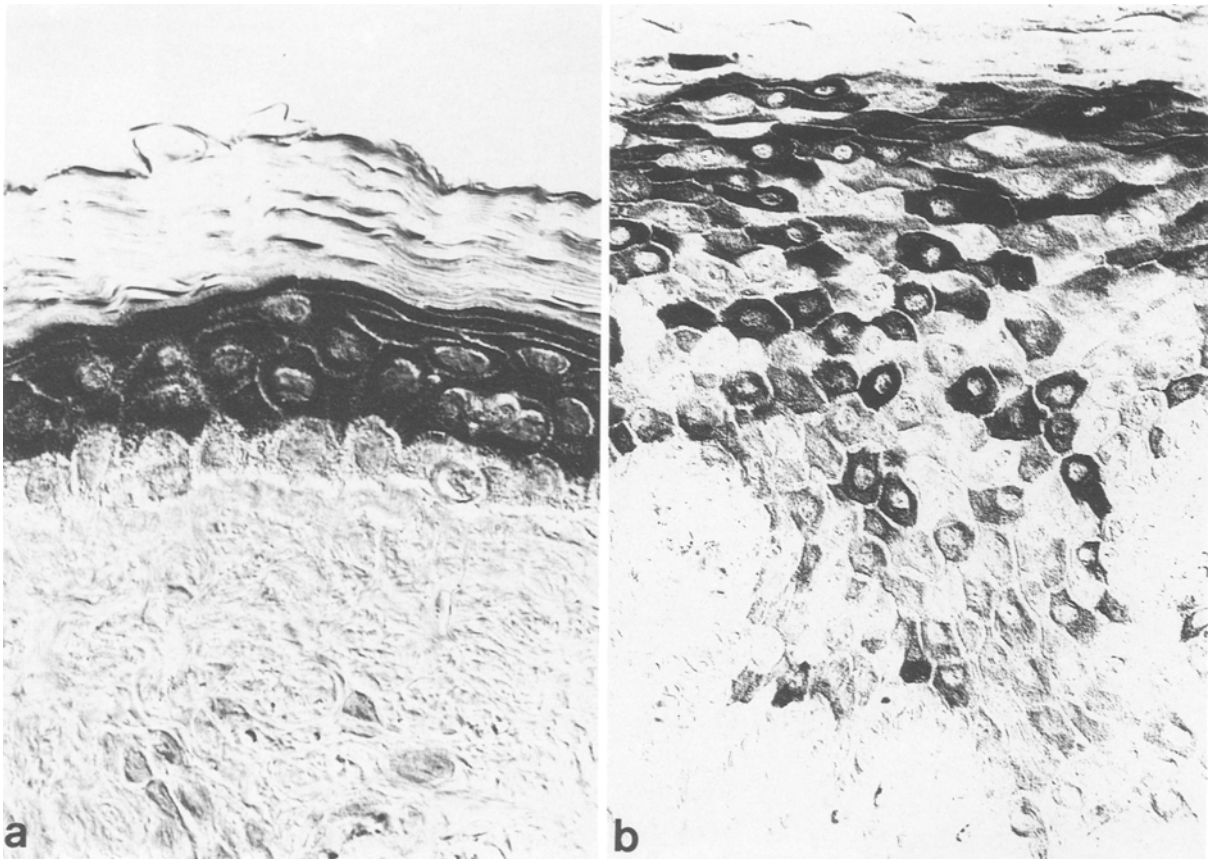

Fig. 2a, b. Immunoperoxidase staining using SK1. In human normal epidermis (**a**) and gingival epithelium, type II oral mucosa (**b**) the staining is specific for suprabasal layers. The irregular positivity of gingiva may be referred to the parakeratinization, while the homogeneous positivity of epidermis is characteristic of orthokeratinized epithelium (interference-contrast optic). **a** ×875; **b** ×350

Table 2. Staining results with monoclonal antibodies SK1 and A53-B/A2 on different types of human normal oral mucosa

	SK1		A53-B/A2	
	B	S	B	S
Type I	−	−/+	+	−
Type II	−	+	−	−
Type III	−	+	−	−

B, Basal layer; S, suprabasal layers; −, negative; −/+, weak and/or irregular; +, strong and regular

cellulose sheets were incubated with undiluted hybridoma supernatant and, after washings, incubated with peroxidase-labelled anti-mouse immunoglobulins. The specificity of SK1 was finally revealed using 4-chloro-1-naphtol as substrate.

Table 3. Staining results with monoclonal antibody SK1 on lesions from different types of human oral mucosa

Case	No	Type I		Type II		Type III	
		B	S	B	S	B	S
Leukoplakia	(14)	−	−/+	−	+	−	+
Leukoplakia with dysplasia	(6)	−	+	−	−/+	−	−/+
Squamous papilloma	(9)	−	−	−	−/+	−	−/+
Squamous cell carcinoma							
G1	(4)		+		+		ND
G2	(5)		−		−		−
G3	(3)		−		−		ND

B, Basal layer; S, suprabasal layers; ND, not done, case not available; −, negative; −/+, weak and/or irregular; +, strong and regular

Results

In all cases, review of haematoxylin-eosin-stained sections confirmed the initial diagnosis. In Table 1 we report the distribution of pathological lesions according to the location and the type of mucosa. Oral lesions comprised 20 cases of nodular leukoplakia (6 showed from mild to severe dysplasia), 9 cases of squamous papilloma and 12 cases of invasive squamous cell carcinoma. By the term leukoplakia, according to the definition given by the WHO Centre (1978), we indicate "a white patch or plaque which cannot be characterized clinically or pathologically as any other condition". The histological features are represented by more or less hyperplastic epithelium with hyper-ortho- and para-keratosis. In the

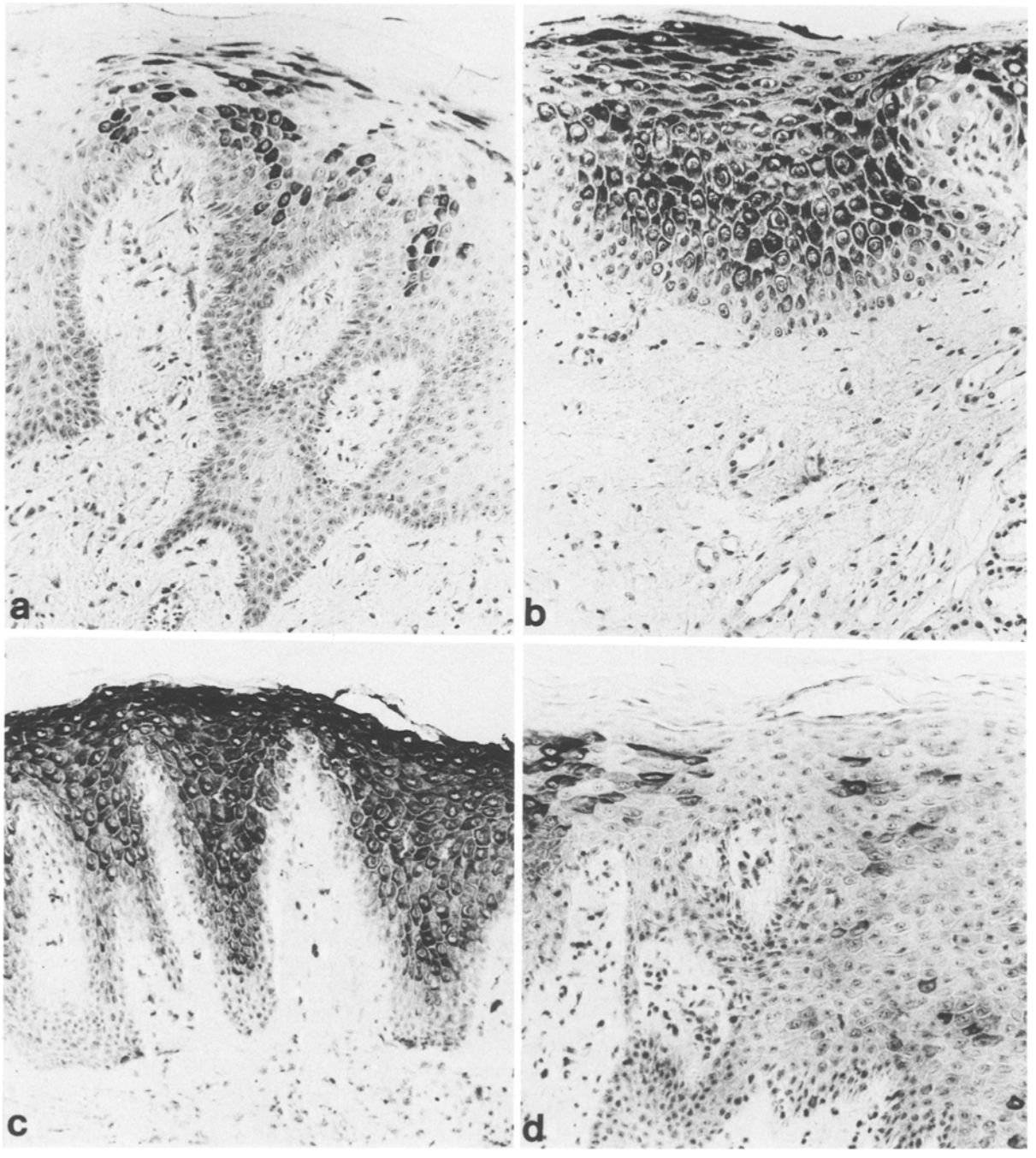

Fig. 3a–d. Immunoperoxidase staining using SK1. Leukoplakia: in type I mucosa SK1 stains suprabasal layers (**a**) but shows greater intensity in presence of dysplasia (**b**). In type II mucosa the staining of suprabasal layers is more evident in absence of dysplasia (**c**) than in presence of dysplasia (**d**). **a–d** × 175

chorion we find a chronic, prevalently lymphocytic, inflammatory infiltrate. The cases of carcinoma were subdivided according to the WHO classification: G1, 4 cases; G2, 5 cases; G3, 3 cases (Wahi et al. 1971). Control cases do not present any alteration with regard to the epithelial component.

The result of immunoblotting is shown in Fig. 1. None of the four major cytokeratin polypeptides expressed in human cultured keratinocytes (Bowden et al. 1987) is recognized by SK1. In contrast, the monoclonal antibody recognizes a polypeptide of 68 kDa quantitatively less present in the extract of keratinocytes. The immunohistochemical investigation performed on human normal tissues showed that this antibody stains the suprabasal layers of human epidermis and keratinized epithelia (Fig. 2a, b), with different patterns reflecting the different types of differentiation (ortho-versus parakeratinization). Other epithelial and non-epithelial tissues were negative. From these data we deduce that SK1 is specific for cytokeratin 1.

The study carried out on the 6 control cases using monoclonal SK1 showed an exclusively suprabasal stain-

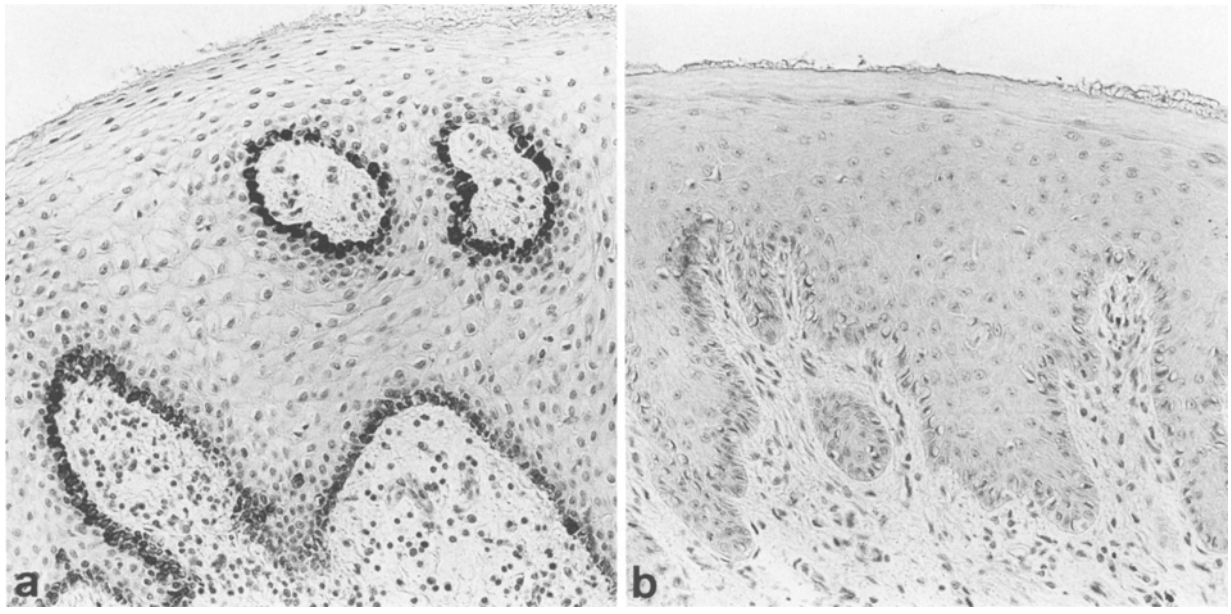

Fig. 4a, b. Immunoperoxidase staining using A53-B/A2. The positivity on normal human vestibular mucosa, type I (**a**) is confined to the basal layer, whereas it is absent on gingival mucosa, type II (**b**). **a, b** ×175

ing. This was particularly intense in type II (Fig. 2b) and with some irregularity in type III mucosa. However, in type I mucosa it was perceivable only occasionally, in scattered cells (Table 2).

In pathological samples (Table 3), SK stained the suprabasal layers in cases of leukoplakia in all types of mucosa, but in type I mucosa the intensity of the staining was greater in the presence of dysplasia (Fig. 3a, b). In contrast, in type II and type III mucosa the staining of suprabasal layers was more intense in the absence of dysplasia (Fig. 3c, d).

Squamous papillomas in type I mucosa are SK1-negative, whereas in the other types of mucosa they show irregular positivity in suprabasal layers. Among the cases of squamous cell carcinoma, only well-differentiated (G1) cases are SK1-positive, while the other cases (G2 and G3), in all types of mucosa, give negative immunostaining (not shown).

Using the A53-B/A2 antibody on normal oral mucosa we always obtained highly regular staining of the basal layers in the regions of non-keratinized mucosa, whereas the other types of mucosa were negative (Table 2; Fig. 4a, b). With the same antibody the same staining of basal layers was seen in the cases of leukoplakia in the type I mucosa. In the presence of dysplasia, irregular positivity appeared in suprabasal layers of type I mucosa (Fig. 5a, b) and even in basal layers of type II and III mucosa (Fig. 5c). The cases of squamous papilloma in type I mucosa showed an intense and regular positivity at the basal level; even the suprabasal layers were irregularly and weakly stained (Fig. 5d). The same lesion on type II and type III mucosa was, in contrast, totally A53-B/A2-negative (not shown). Well-differentiated squamous cell carcinoma is always negative and the less differentiated types (G2 and G3), particularly in type I mucosa, are highly stained (Table 4; Fig. 5e).

Discussion

It is well known that cytokeratin 1 is expressed in the keratinized epithelia (Quinlan et al. 1985; Sun et al. 1985) or in those epithelia which keratinize as a result of vitamin A deficiency (Fuchs and Green 1981). The expression of cytokeratin 1 is suppressed when retinoic acid is added to normal epidermal cultures (Kopan et al. 1987; Regnier and Darmon 1989) and reduced in epidermal psoriatic cells (Van Neste et al. 1988), and it is clear that as a marker of a keratinization programme of the epithelial cell it does not belong exclusively to the epidermis. In particular, there is biochemical and immunohistochemical evidence of the presence of this polypeptide in differently keratinized regions of the oral mucosa (Ouhayoun et al. 1985; Morgan et al. 1987a; Dhouailly et al. 1989; Reibel et al. 1989).

In all these cases the distribution of cytokeratin 1 is limited to the suprabasal cells. In human oral mucosa the suprabasal localization and the site-specific distribution of cytokeratin 1 were confirmed using our monoclonal antibody SK1. In fact, only in the keratinized mucosa of masticatory type (II) and in particular areas of specialized mucosa of the dorsal tongue (III) did we detect a strong and regular immunostaining. From the epidermal point of view, this proves the completion of the differentiation programme of these oral mucosa regions, in contrast to the regions of non-keratinized mucosa.

Our data indicate that the immunostaining of leukoplakia in type II, but also in type III oral mucosa, gives results which are quite similar to those obtained on normal tissues except in cases with dysplasia. Here the positivity with SK1 is less intense and more irregular. This may suggest a decreasing differentiation capacity concomitant with epithelial dysplasia. However, surprising-

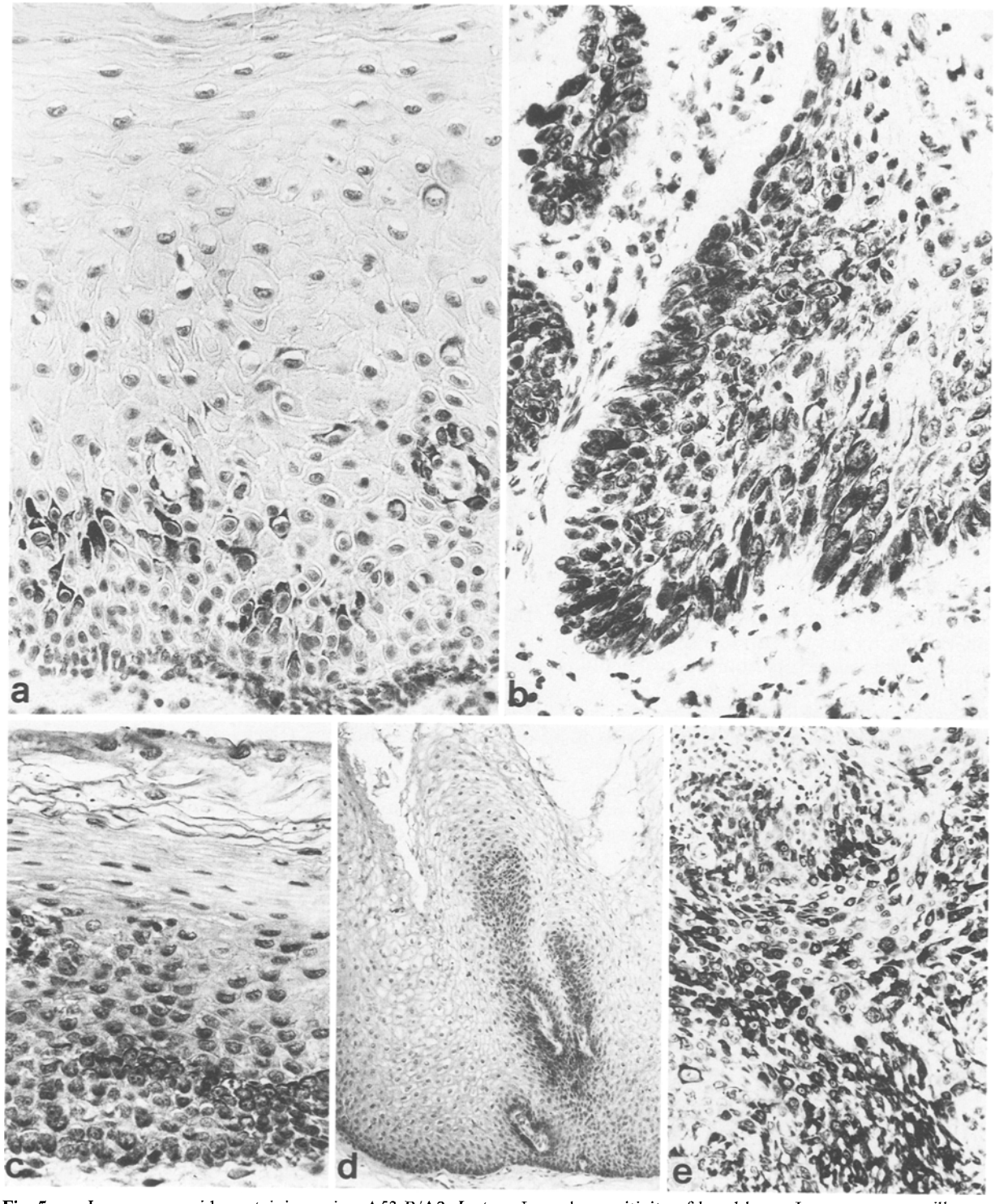

Fig. 5a–e. Immunoperoxidase staining using A53-B/A2. In type I mucosa (soft palate, vestibular mucosa), cases of leukoplakia with mild (**a**) and particularly with severe (**b**) dysplasia show irregular positivity in basal and suprabasal layers. In type II mucosa (hard palate) the one case of leukoplakia with dysplasia (**c**) shows irregular positivity of basal layer. In squamous papilloma of soft palate (**d**) the positivity is evident and regular in basal layers and irregular in suprabasal layers. In squamous cell carcinoma, G3, of type I mucosa (vestibular mucosa) neoplastic cells are intensely positive (**e**). **a–c** ×350; **d** ×88; **e** ×175

ly, in cases of leukoplakia with dysplasia originating in non-keratinized mucosa, we find an inverse phenomenon. There is an increasing positivity of SK1 staining comparable to that found in keratinized regions. This may be due to hyperdifferentiative metaplasia associated with dysplasia in an incompletely keratinized epithelium. This epithelium, which retains a wider potential, may keratinize in association with the dysplastic phenomenon, which itself interferes with the differentiation programme. This fact seems to indicate that the differentia-

Table 4. Staining results with monoclonal antibody A53-B/A2 on lesions from different types of human oral mucosa

Case	No	Type I		Type II		Type III	
		B	S	B	S	B	S
Leukoplakia	(14)	+	−	−	−	−	−
Leukoplakia with dysplasia	(6)	+	−/+	−/+	−	−/+	−
Squamous papilloma	(9)	+	−/+	−	−	−	−
Squamous cell carcinoma							
G1	(4)	−		−		ND	
G2	(5)	−/+		−/+		−/+	
G3	(3)	+		−/+		ND	

B, Basal layer; S, suprabasal layers; ND, not done, case not available; −, negative; −/+, weak and/or irregular; +, strong and regular

tion programme of the cell involved in a pre-neoplastic phenomenon may be important in conditioning different intermediate stages of reaction to similar oncogenic stimuli, confirming the observation that the behaviour of oral neoplasms may differ in relation to their location (Wahi et al. 1971). The different distribution of SK1 positivity on the various types of mucosa, in contrast, is no longer evident in neoplasia. In all the cases analysed the maximum intensity is observed in well-differentiating carcinoma (G1), whereas positivity rapidly disappears as the malignant grade increases. The results we obtained agree with those described by other authors using biochemical techniques or different antibodies (Löning et al. 1980, 1982; Löning and Burkhardt 1982; Morgan et al. 1987b).

With regard to the data gathered on the presence of cytokeratin 19 using monoclonal A53-B/A2, previous studies have shown that this cytokeratin is present in the basal layers of non-keratinized oral mucosa (Morgan et al. 1987a; Lindberg and Rheinwald 1989). It is completely absent in keratinized oral mucosa. Our results are in accordance with those already reported.

In benign pathology, particularly in leukoplakia without dysplasia, we could not find any modifications in immunostaining. The presence of dysplasia in type II and III mucosa regions is, however, characterized by the appearance of an irregular positivity of basal layers. In the presence of malignancy, the immunostaining is increasingly intense in the various grades of carcinoma, from G1 to G3. In type I mucosa, an increasing positive staining with SK1 in suprabasal layers is particularly evident in cases of leukoplakia with severe dysplasia. These data confirm the finding that the expression of the cytokeratin 19 is related to pre-neoplastic situations (Lindberg and Rheinwald 1989). We cannot exclude that, together with leukoplakia, even inflammation can be responsible for the positivity of this cytokeratin, as reported by Bosch et al. (1989). However, we believe that the expression of cytokeratin 19 reaches the maximum intensity in association with the highest levels of dysplasia. Even in some cases of squamous papilloma which have their origin in non-keratinized mucosa, we have also found an increasing presence of cytokeratin 19 detectable in suprabasal layers. Moreover, in cases of malignancy, cytokeratin 19 seems to correlate with the differentiation of the tumour as previously reported (Morgan et al. 1987b).

In conclusion, the immunolocalization of cytokeratins 1 and 19 on human oral mucosa allows us to confirm the topographic differences in oral cavity epithelium. In any case, we can state that the cytokeratin patterns detected in type II and type III mucosa are substantially similar to one another and are very different from those of the type I mucosa. This concerns a different immunostaining in the case of dysplasia on leukoplakia. Our findings also indicate that malignancy is concomitant with both a progressive disappearance of cytokeratin 1 and a progressive increase of 19.

Acknowledgements. The authors are grateful to Massimo Bugnoli for providing the monoclonal antibody and Catherine Mallia for editing the manuscript.

References

Battifora H, Kopinski M (1986) The influence of protease digestion and duration of fixation on the immunostaining of keratins. A comparison of formalin and ethanol fixation. J Histochem Cytochem 34:1095–1100

Bosch FX, Ouhayoun JP, Bader BL, Collin C, Grund C, Lee I, Franke WW (1989) Extensive changes in cytokeratin expression patterns in pathological affected human gingiva. Virchows Arch [B] 58:59–77

Bowden PE, Stark HJ, Breitkreuz D, Fusenig NE (1987) Expression and modification of keratins during terminal differentiation of mammalian epidermis. Curr Top Dev Biol 22:35–68

Cianfriglia M, Armellini D, Massone A, Mariani M (1983) A simple immunization protocol for high frequency production of soluble antigen-specific hybridomas. Hybridoma 2:451–456

Clausen H, Vedtofte P, Moe D, Dabelsteen E, Sun T-T, Dale B (1986) Differentiation-dependent expression of keratins in human oral epithelia. J Invest Dermatol 86:249–254

Dhouailly D, Xu C, Manabe M, Schermer A, Sun T-T (1989) Expression of hair-related keratins in a soft epithelium: subpopulations of human and mouse dorsal tongue keratinocytes express keratin markers for hair-, skin- and esophageal-types of differentiation. Exp Cell Res 181:141–158

Eichner R, Bonitz P, Sun T-T (1984) Classification of epidermal keratins according to their immunoreactivity, isoelectric point and mode of expression. J Cell Biol 98:1388–1396

Ermich T, Schulz J, Schumann D (1988) Pattern of oral cytokeratins. I. SDS-electrophoretic analysis of the frequency of cytoskeletal keratins in the normal human mucosa of the mouth. Biomed Biochim Acta 8:737–742

Franke WW, Schiller DL, Moll R, Winter S, Schmid E, Engelbrecht I, Denk H (1981) Diversity of cytokeratins. Differentiation specific expression of cytokeratin polypeptides in epithelial cells and tissues. J Mol Biol 153:933–959

Fuchs E, Green H (1981) Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell 25:617–625

Fuchs EV, Coppock SM, Green FH, Cleveland DW (1981) Two distinct classes of keratin genes and their evolutionary significance. Cell 27:75–84

Fuchs E, Tyner AL, Giudice GJ, Marchuk D, Ray Chaudury A, Rosenberg M (1987) The human keratin genes and their differential expression. Curr Top Dev Biol 22:5–34

Kasper M, Stosiek P, Typlt H, Karstens U (1987) Histological

evaluation of three new monoclonal anti-cytokeratin antibodies. 1. Normal tissues. Eur J Cancer Clin Oncol 23:137–147

Kopan R, Traska G, Fuchs E (1987) Retinoids as important regulators of terminal differentiation: examining keratin expression in individual epidermal cells at various stages of keratinization. J Cell Biol 105:427–440

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lazarides E (1982) Intermediate filaments: a chemically heterogeneous, developmentally regulated class of proteins. Annu Rev Biochem 51:219–250

Lindberg K, Rheinwald JG (1989) Suprabasal 40 kd keratin (K19) expression as an immunohistologic marker of premalignancy in oral epithelium. Am J Pathol 134:89–98

Löning T, Burkhardt A (1982) Dyskeratosis in human and experimental oral precancer and cancer. An immunohistochemical and ultrastructural study in men, mice and rats. Arch Oral Biol 27:361–366

Löning T, Staquet MJ, Thivolet J, Seifert G (1980) Keratin polypeptides distribution in normal and diseased human epidermis and oral mucosa. Immunohistochemical study on unaltered epithelium and inflammatory, premalignant and malignant lesions. Virchows Arch [A] 388:273–288

Löning T, Viac J, Caselitz J, Thivolet J, Seifert G (1982) Comparative investigation of keratin filaments in normal tissues and tumours of skin, oral mucosa, salivary glands and thymus. Pathol Res Pract 175:256–265

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratin polypeptides: patterns of expression of specific cytokeratins in normal epithelia, tumors and cultured cells. Cell 31:11–24

Morgan PR, Leigh IM, Purkis PE, Gardner ID, Van Muijen GNP, Lane EB (1987a) Site variation in keratin expression in human oral epithelia. An immunohistochemical study of individual keratins. Epithelia 1:31–43

Morgan PR, Shirlaw PJ, Johnson NW, Leigh IM, Lane EB (1987b) Potential applications of anti-keratin antibodies in oral diagnosis. J Oral Pathol 16:212–222

Nakai M, Mori M (1986) Immunohistochemical distribution of monoclonal antibodies against keratin in papillomas and carcinomas from oral and nasopharyngeal regions. Oral Surg 62:292–302

Ouhayoun JP, Gosselin F, Forest N, Winter S, Franke WW (1985) Cytokeratin patterns of human oral epithelia: differences in cytokeratin synthesis in gingival epithelium and the adjacent alveolar mucosa. Differentiation 30:123–129

Quinlan RA, Schiller DL, Hatzfeld M, Achtstatter T, Moll R, Jorcano JL, Magin TM, Franke WW (1985) Patterns of expression and organization of cytokeratin intermediate filaments. In: Wang E, Fishman D, Liem RKH, Sun T-T (eds) Intermediate filaments, vol 455. The New York Academy of Sciences, New York, pp 282–306

Regnier M, Darmon M (1989) Human epidermis reconstructed in vitro: a model to study keratinocyte diferentiation and its modulation by retinoic acid. In Vitro Cell Dev Biol 25:1000–1008

Reibel J, Clausen H, Dabelsteen E (1985) Staining patterns of human premalignant oral epithelium and squamous cell carcinomas by monoclonal anti-keratin antibodies. Acta Pathol Microbiol Immunol Scand Sect A 93:323–330

Reibel J, Clausen H, Dale B, Thacher SM (1989) Immunohistochemical analysis of stratum corneum components in oral squamous epithelia. Differentiation 41:237–244

Schroeder HE (1981) Differentiation of human oral stratified epithelia. Karger, Basel

Steinert PM, Steven AC, Roop DR (1985) The molecular biology of intermediate filaments. Cell 42:411–419

Sun T-T, Tseng SCG, Huang AJW, Cooper D, Schermer A, Lynch MH, Weiss R, Eichner R (1985) Monoclonal antibody studies of mammalian epithelial keratins: a review. In: Wang E, Fishman D, Liem RKH, Sun T-T (eds) Intermediate filaments, vol 455. The New York Academy of Sciences, New York, pp 307–329

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Van Neste DJJ, Staquet MJ, Leroy BP, De Coster WJ (1988) Distribution pattern of psoriatic keratoblasts: computer-assisted image-analysis for combined evaluation of DNA synthesis and expression of 67kD keratin polypeptides in the epidermis of stable plaques of psoriasis. J Invest Dermatol 90:382–386

Vigneswaran N, Peters KP, Hornstein OP, Haneke E (1989) Comparison of cytokeratin, filaggrin and involucrin profiles in oral leukoplakia and squamous carcinomas. J Oral Pathol Med 18:377–390

Wahi PN, Cohen B, Luthra UK, Torlon H (1971) Histological typing of oral and oropharyngeal tumours. World Health Organization, Geneva

WHO collaborating reference centre for oral precancerous lesions (1978) Definition of leukoplakia and related lesions: an aid to studies on oral precancers. Oral Surg 46:517–539