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Original Article

Characterization of cytokeratin patterns in the developing human tongue

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ABSTRACT The characterization of cytokeratin (CK) in adult oral mucosa and developing teeth have been well documented in human. Cytokeratin distribution in developing oral mucosa has not yet been described. The aim of this study was to identify the expression of CK in human fetal tongue (week 10 to week 23) and to correlate the results with morphological maturation. Simple epithelial CK are expressed in all cell layers during the early stages, essentially in peridermal cells. From the 14th week, CK 18 is present only in the taste buds, making this polypeptide a reliable marker for this sensory organ. CK 4 and 13 are expressed from the 10th to the 23rd week by both ventral and dorsal lingual epithelia. Terminal differentiation keratins (CK 1, 2 and 10-11) can only be detected immunohistochemically at the 14th week in some cells on the external surface of some papillae. The number of these papillae and positive cells increase at the 19th and 23rd weeks. The terminal differentiation markers are expressed several weeks earlier than the formation of a well-distinguished keratinized layer.

KEY WORDS: *cytokeratins, cytoskeleton, oral epithelia, tongue, oral embryology*

Introduction

The lingual epithelium has two embryonic origins: the base region is endodermal, and the remainder is ectodermal (Provenza and Seibel, 1986). The lingual simple epithelium of early embryonic life gradually becomes stratified squamous. The first lingual papillae that appear on the dorsal surface of the tongue are the caliciform papillae, which develop as early as the 9th to 10th week (Yamasaki and Takahashi, 1982; Provenza and Seibel, 1986), and the foliate papillae develop over the next few weeks (Milaire, 1980). The fungiform papillae appear in the 11th week (Milaire, 1980), whereas the filiform papillae are the last to develop (Provenza and Seibel, 1986). Taste buds appear between the 7th and the 8th weeks (Sperber, 1981). Their locations determine the future sites of the fungiform and caliciform papillae (Milaire, 1980; Provenza and Seibel, 1986). During intra-uterine life the epithelium of fungiform papillae contains only one taste bud (Arvidson, 1979); it is only after birth that several buds may be found on these papillae. The taste buds become functionally mature around the 15th week (Bradley and Stern, 1967).

Cytokeratins are the intermediate filament proteins of epithelial cells. They form a complex family of 20 different polypeptides in human tissues, with molecular weights ranging from 40 to 68 kD (Franke *et al.*, 1981, 1984; Moll *et al.*, 1982a, 1990; Quinlan *et al.*, 1985; Sun *et al.*, 1984). Different epithelia within the same species express different combinations of cytokeratin polypeptides (Doran *et al.*, 1980; Tseng *et al.*, 1982; Heid *et al.*, 1988), but there is always at least one acidic (type I) and one basic (type II) protein in the cytokeratin subunit (Fuchs *et al.*, 1981, 1987; Sun *et al.*, 1984).

Cytokeratins appear to be excellent markers for different patterns of epithelial differentiation. Simple epithelia express two to four of the smaller cytokeratins (CK 7, 8, 18 and 19). Stratified squamous epithelia are characterized by the presence of larger polypeptides, mainly CK 1 to 6 and 9 to 17, but the profile varies with the differentiation program. For example the epidermal (skin)-type cells contain CK 1, 2 and 10-11, while in the esophageal type, cells express mainly CK 4 and 13 (Sun *et al.*, 1984).

The distribution of cytokeratins in adult oral mucosa is well documented (Ouhayoun *et al.*, 1985; Morgan *et al.*, 1986, 1987; Shabana *et al.*, 1989; Sawaf *et al.*, 1990), and changes in the cytokeratin pattern in developing human teeth have recently been described (Lesot *et al.*, 1982; Nishikawa *et al.*, 1988; Kasper *et al.*, 1989; Pelissier *et al.*, 1990). However, to our knowledge, the changes in cytokeratin profiles in the developing human tongue have not been published. We have identified these changes and correlated the results with the morphological maturation of the lingual epithelium during the development of the tongue.

Abbreviations used in this paper: CK, cytokeratin(s); NEPHGE, non-equilibrium pH gradient electrophoresis; EDTA, ethylenediamine-tetraacetic acid.

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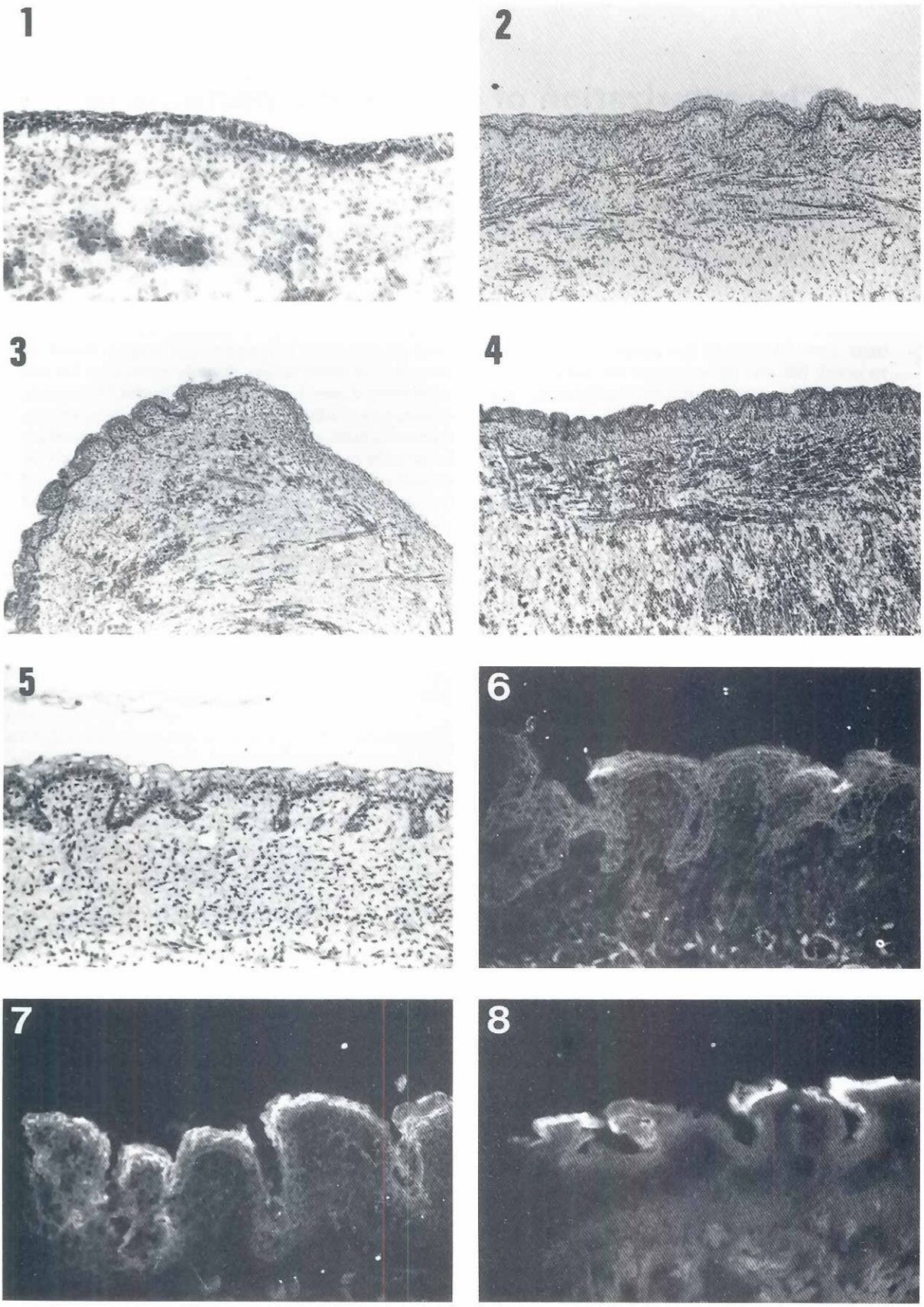
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Results

Histology

10th week

The ventral tongue epithelium appeared thin (Fig. 1), with three epithelial layers: a deeply stained small basal cell layer, a superficial layer of lightly stained large, flattened cells and an intermediate cell layer.

The epithelial-connective tissue junction was flattened. The lamina propria contained loose collagen fibers and was rich in mesenchymal cells. At this age, the morphological appearance of the dorsal and ventral epithelia were similar, except for some primitive fungiform papillae which slightly projected from the dorsal surface (Fig. 2).

14th week

The dorsal lingual epithelium showed numerous papillae, most of them having fungiform appearance (Figs. 3, 4 and 5). The ventral epithelium was still thin. The lamina propria was histologically like that of the 10-week-old fetus.

The lamina propria showed evidence of collagen fiber synthesis, as indicated by the green stain in sections stained with Masson trichrome. The muscles were more developed and nerve fibers could be distinguished. Taste buds could not be identified histologically at this age.

19th week

The epithelial cell layers of both dorsal and ventral surfaces were thicker. The mesenchymal tissue was better developed, with well-structured muscles, nerves and blood vessels. The lingual papillae were large and inclined backward towards the pharynx.

23rd week

A keratinized layer could be seen on the external surfaces of the papillae. Taste bud-like structures were present in the epithelium of some fungiform papillae. Most papillae had a flattened top, although some had begun to show elongated tips. The connective tissue was rich in collagen fibers and mesenchymal cells. The muscle fibers were dense and blood vessels and nerves could be identified between the bundles.

Immunohistochemistry

10th week

Both dorsal and ventral epithelia of the tongue were negative to antibodies specific for terminal differentiation cytokeratins, CK 1, 2 and 10-11, (EE21.6, RKSE60 and Kk8.60). In contrast, these epithelia were strongly labeled by antibodies specific for CK 4 and 13 of non-keratinizing stratified squamous epithelia. These antibodies (6B10, 1C7 and F2III) marked the suprabasal cell layers. The basal cells and epithelial prolongations, which are the precursors of minor salivary glands, were negative. Simple epithelial cytokeratins (CK 8, 18 and 19) were detected in the tongue lining epithelium. The superficial cells were more strongly labeled than the deeper cell layers and the epithelial prolongations, as shown by the reactions with antibodies 42.39.13.1 (specific for CK 8) (Fig. 12), Ks18.174 (against CK 18) (Fig. 11), and Ks19.1 (anti-CK 19). Broad spectrum antibodies did not show similar distributions in the epithelium. Antibodies KL1 and Ks8.12 were moderately positive in the basal and intermediate layers, and strongly reactive with the superficial layer. The antibody Ks13.1 (specific for CK 13, and to a lesser extent, CK 14 and 17) showed intense reaction in the suprabasal cells. Finally, Ks1-8 (antibody which reacts with basic cytokeratins) reacted in all cell layers uniformly.

14th week

Terminal differentiation keratins were not detected by the antibodies EE21.6, Kk8.60 and RKSE60 in either dorsal or ventral epithelia. However, individual cells in the external epithelium of certain lingual papillae were labeled, particularly with Kk8.60 (Fig. 6). Cytokeratins 4 and 13 were not detected in the basal cells of papillary, interpapillary and ventral epithelia. They were expressed in all suprabasal cells (Figs. 15 and 16).

The antibody specific for CK 19 reacted with all epithelial layers in the dorsal and ventral epithelia. In contrast, CK 18 was only detected in epithelial islands in the mesenchymal tissue, corresponding to minor salivary glands (Fig. 14) and in small structures in the top of some fungiform papillae (Fig. 13). These structures may be taste buds. Cytokeratin 8 was still detectable in the more superficial cell layer of the lingual epithelia. Antibodies KL1 and Ks8.12 labeled the basal and suprabasal cell layers, although labeling was stronger in suprabasal cells. Pan 1-8 gave a similar distribution pattern.

19th week

There was a marked increase in the number of cells containing terminal differentiation markers on the external surfaces of papillae. The ventral and interpapillary epithelia were reactive for cytokeratins 4 and 13 in the suprabasal cell layers. In the papillary epithelium, CK4 was expressed in the suprabasal cell layers; CK13 was equally expressed in these layers, but was absent from the tip of certain papillae (Figs. 9 and 10). These negatively reacting sites

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Fig. 1. Histological section of the ventral surface of the fetal tongue at 10 weeks. *There are three epithelial cell layers – a basal, spinous and periderm. The mesenchymal tissue includes immature muscles and mesenchymal cells. (x100).*
Fig. 2. Dorsal surface of the fetal tongue (10 weeks) showing some fungiform papillae. *The interpapillary epithelium appears flattened (x40).*
Fig. 3. Sagittal section of the tongue (14 weeks). *The dorsal surface is covered with numerous papillae, whereas the ventral surface lacks these papillary structures. (x40).*
Fig. 4. The dorsal surface of human fetal tongue (14 weeks). *All the papillae at this age resemble fungiform papillae rather than the filiform variety. The lingual musculature is more developed than at 10 weeks. (x40).*
Fig. 5. The dorsal surface of a 14-week-old fetal tongue. *The papillae have all the morphological features of fungiform papillae. (x100).*
Fig. 6. The reaction of Kk8.60 (antibody to CK 10 and 11) in the dorsal tongue epithelium (14 weeks). *Note the positive cells in the top of the fungiform papillae. (x100).*
Fig. 7. Fetal dorsal tongue at 23 weeks. *Positive reaction in the upper part of the papillae to antibody EE21.6 (anti-CK 1, 2, 10 and 11) (x100).*
Fig. 8. Dorsal surface of tongue of 23-week-old fetus. *The filiform papillae are inclined towards the pharynx. The top of these papillae show a positive reaction for antibody RKSE60, indicating keratinized epithelia. (x100).*

for CK13 correspond to positive sites for terminal differentiation markers. The distribution of cytokeratin 19 was the same as in the 14-week-old fetuses, while CK8 immunoreactivity became very weak and cytokeratin 18 was totally absent from the stratified squamous epithelia, but remained in the taste buds and salivary gland epithelia. All suprabasal layers were positive with antibodies KL1 and Ks8.12. Antibody Ks13.1 faintly labeled the basal cell layer and reacted strongly with all other epithelial cell layers.

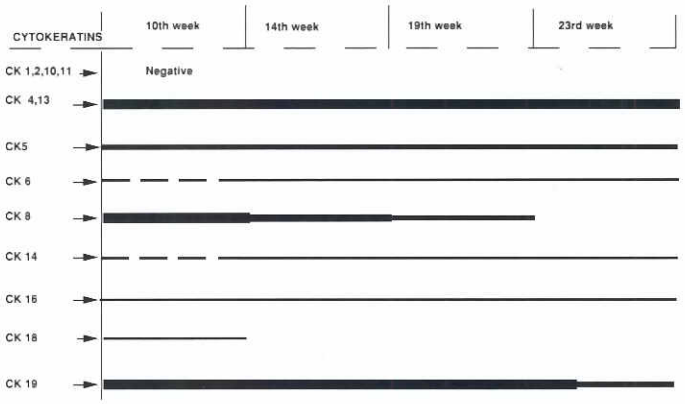
TABLE I

THE PRIMARY MONOCLONAL ANTIBODIES USED

Antibody	Specificity	Source	Reference
KL1	Cytokeratins n° 1,2,5, 6,7,8,9,10,11 and 19	Immunotech Luminy-Marseille France	Viac *et al.* (1983) and personal unpublished data
Ks1-8	Cytokeratins n° 1 to 8	Progen - Biotechnics Heidelberg, FRG	
EE21-6	Cytokeratins n° 1,2,10,11	Kindly provided by G. Serres-Lab. Exploration biologique cellulaire Toulouse - France	Personal unpublished data
RKSE60	Cytokeratin n° 10	Eurobio	Ramaekers *et al.* (1983)
Kk8-60	Cytokeratins n° 10,11	Bio-Yeda	Huszar *et al.* (1986)
6B10	Cytokeratin n° 4	Kindly provided by W.W. Franke, German Cancer Res. Center Heidelberg	Van Muijen *et al.* (1986)
Ks13-1	Cytokeratins n° 13 (14,17)	Progen - Biotechnics Heidelberg	Moll *et al.* (1988)
F2 III	Cytokeratin n° 13	Kindly provided by W.W. Franke German Cancer Res. Center Heidelberg	
1C7	Cytokeratin n° 13	Kindly provided by W.W. Franke German Cancer Res. Center Heidelberg	Van Muijen *et al.* (1986)
Ks8-12	Cytokeratins n° 13,16 (and others ?)	Bio-Yeda	Huszar *et al.* (1986)
Kb37	Cytokeratins of basal cells	Sigma, Saint-Louis, MO, USA.	
42-39-13-1	Cytokeratin n° 8	Kindly provided by W.W. Franke German Cancer Res. Center Heidelberg	
Ks18.174	Cytokeratin n° 18	Progen - Biotechnics Heidelberg	Moll *et al.* (1988)
Ks19-1	Cytokeratin n° 19	Progen	Karsten *et al.* (1985)

TABLE II

DYNAMIC REPRESENTATION OF COMBINED BIOCHEMICAL AND IMMUNOHISTOCHEMICAL DATA IN DORSAL (EXCEPT FOR TOP AND TIP OF PAPILLAE) AND VENTRAL LINGUAL EPITHELIA OF FETAL HUMAN TONGUE, FROM 10 TO 23 WEEKS



23rd week

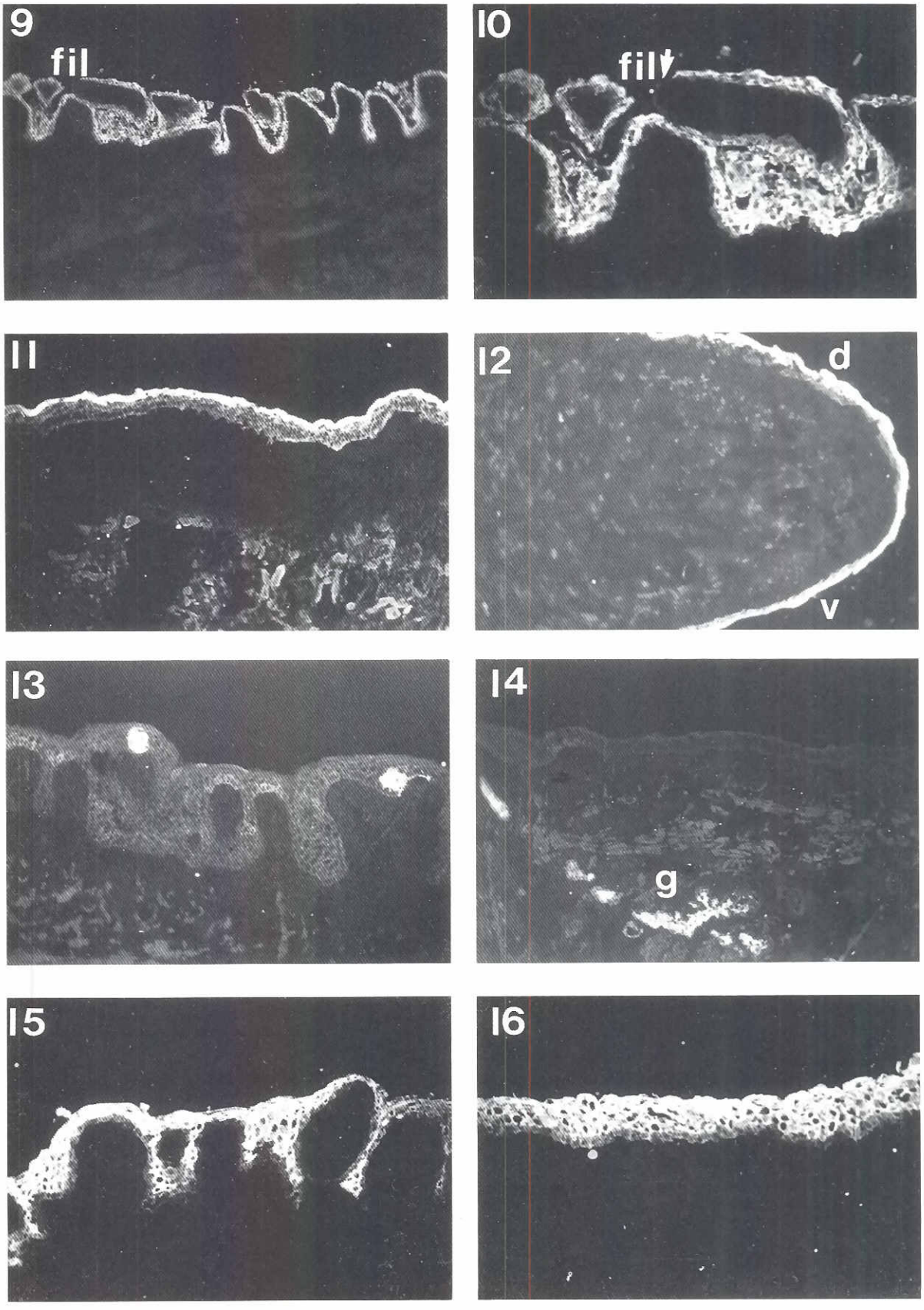
All the antibodies specific for terminal differentiation keratins reacted positively with the external surfaces of the lingual papillae (Figs. 7 and 8). All other lingual epithelia were negative for these differentiation markers. The monoclonal 6B10 (anti-CK4), 1C7 and F2 III (anti-CK 13) were positive in both ventral and dorsal epithelia, and always reacted with the suprabasal cell layers. However, several lingual papillae were negative in their external surfaces. Cytokeratin 19 was detected in all lingual epithelia, although the reaction was less strong than at the earlier stages of developement. At this age, the epithelial reaction to anti-CK 8 was greatly reduced or even negative in the lining and papillary epithelia. There was no immunostaining for CK 18 in these epithelia. The antibodies KL1 and Ks8.12 were always strongly positive in the suprabasal cell layers of all epithelia. Similarly, Ks13.1 stained all suprabasal cell layers, though the basal cells stained weakly. Antibody Ks1-8 reacted with all strata of the epithelium equally well in all regions of the tongue.

Biochemistry

10th week

The cytokeratins were extracted from the whole body of the tongue. The two-dimensional gels showed cytokeratins 5, 13 and 19 as major bands and cytokeratin 16 as a minor band. Traces of

Fig. 9. Reaction of filiform papillae (fil) of 19th week fetal tongue with anti-CK4 (Monoclonal 6B10). *Note the absence of labeling in the epithelium covering the external surface and the tips of the papillae (x40).*
Fig. 10. Higher magnification of Fig. 9. *Note the negative reaction in the tip of the papillae (arrow) (x100).*
Fig. 11. The ventral surface of 10-weeks fetal tongue. *Cytokeratin 18 is expressed mainly by the superficial layer of the epithelium (periderm) (x100).*
Fig. 12. Sagital section in 10-weeks fetal tongue stained for cytokeratin 8. *Note the labeling of both dorsal (d) and ventral (v) epithelia. This labeling is more intense in the periderm (x40).*
Fig. 13. Dorsal surface of 14-week-old fetal tongue. *Anti-cytokeratin 18 labeled the taste buds in the fungiform papillae. The surrounding epithelium is almost negative (x100).*
Fig. 14. Ventral surface of the 14-weeks fetal tongue. *The lining epithelium is negative for cytokeratin 18. The only reacting epithelial cells are the salivary gland epithelia (g) present in the mesenchyme (x100).*
Fig. 15. Dorsal surface of a 14-week-old fetal tongue immunostained with anti-CK4 (6B10). *The suprabasal cell layers are heavily labeled. (x100).*
Fig. 16. Ventral surface of 14-week-old fetal tongue immunostained with anti-CK 4 (6B10). *All the suprabasal cell layers were positive. (x100).*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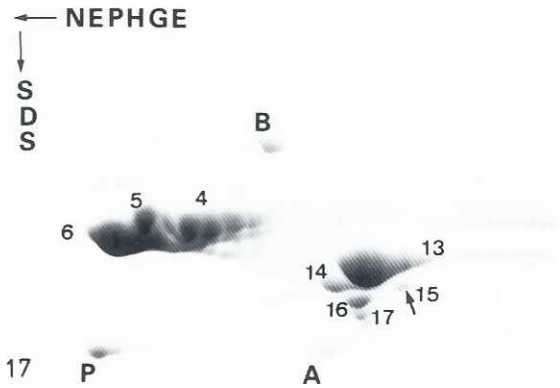

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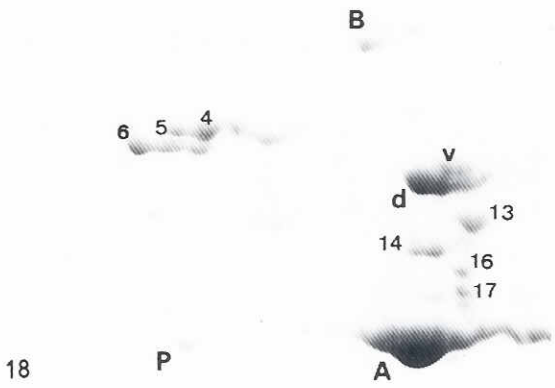

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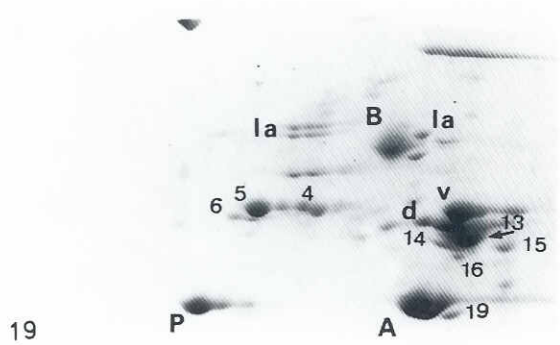

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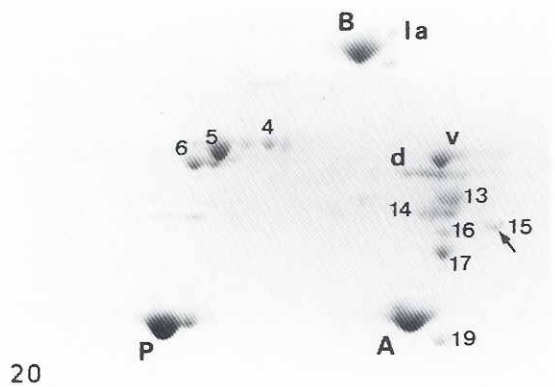

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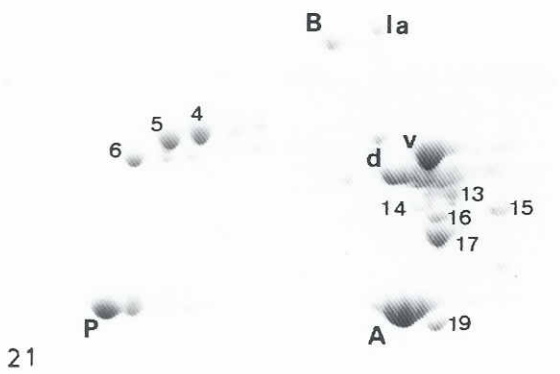

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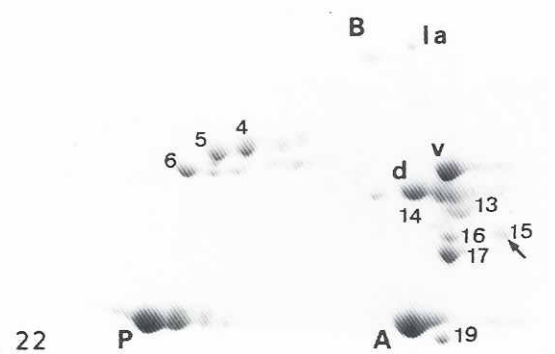

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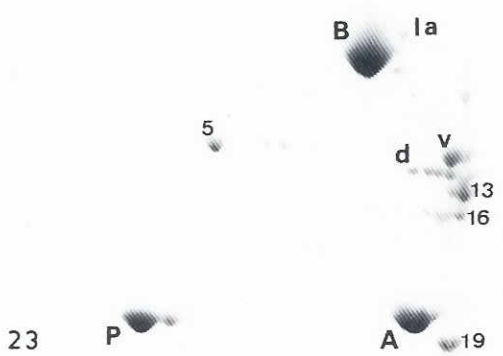

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CK15 were also detectable in overloaded gels. Two bands corresponding to vimentin and desmin were also present (Fig. 23). These intermediate filaments were present on gels for all ages.

14th week

Ventral surface. In addition to CK 5 and 13, the other major bands were CK 4, 6, 16, 17 and 19. The minor bands were cytokeratins 14 and 15 (Fig. 21).

Dorsal surface. The electrophoretic profile of cytokeratins in the dorsal epithelia was identical to that of the ventral surface (Fig. 22).

19th week

Ventral surface. The gels contained heavily stained bands of cytokeratins 5 and 13. The other major cytokeratins were CK 4, 15, and 19, and the minor ones were CK 6, 14 and 16 (Fig. 19).

Dorsal surface. The cytokeratin profile was clearly different from the one of ventral tongue, in that CK 17 produced a major band, while the other bands were the same as those described for the ventral surface (Fig. 20).

23rd week

Ventral surface. The major bands were CK 4, 5, 6, 13, 14 and 16, and the minor bands were CK 15 and 17 (Fig. 17).

Dorsal surface. The electrophoretic pattern differed slightly from that of the ventral surface. The dorsal surface lacked CK 15, but CK 17 was clearly present (Fig. 18).

Discussion

This study is, to our knowledge, the first description of cytokeratin expression during the development of the human fetal tongue.

The development of the human tongue epithelium goes through an initial embryonic stage, up to the 8th week, and a fetal stage which starts in the 9th week and continues to birth. The lingual epithelium at the end of the embryonic stage is made up of 2 cell layers, a basal layer and a periderm, as is the epidermis (Holbrook and Odland, 1975). A third layer of cells appears between the basal layer and the periderm early in the fetal stage. This third layer was seen in the lingual epithelium of 10-week-old fetuses. This is the stratification stage, (Holbrook and Odland, 1975; Dale and Holbrook, 1987). The dorsal surface of the tongue bears only primitive fungiform papillae during this period. The dorsal surface is covered by papillae which more closely resemble the fungiform rather than the filiform by the 14th week. The epithelium is thicker at the 19th week, as a result of an increase in the number of cell layers. The tips and tops of the papillae become keratinized by week 23 and they are surrounded by non-keratinized interpapillary epithelium. Meanwhile, the ventral epithelium shows all the characteristics of a non-keratinized stratified squamous epithelium.

The immunohistochemical studies showed that simple epithelial cytokeratins (CK 8, 18 and 19) are expressed in the superficial layer of both dorsal and ventral epithelia during the early stages of stratification, whereas the basal and intermediate layers contain little of these markers. These large, flattened superficial cells are peridermal cells.

During the embryonic stage, the periderm, which covers the epidermis, reacts positively with antibodies to CK 18 and 19, while the basal cell layer does not react (Moll *et al.*, 1982b). The periderm retains this expression during the stage of epidermal stratification (Dale and Holbrook, 1987). In this study, all epithelial cell layers express CK 19 at the 14th week, while CK 18 remains only in the taste buds, and CK 8 is expressed only in the periderm. It is from this stage of fetal life that CK 18 is detectable only in the taste buds, making it a reliable marker for taste buds from this age up to birth.

At the 19th week, CK 19 is expressed by all epithelial cell layers, whereas CK 8 becomes less and less abundant and finally disappears at the 23rd week. At this stage, the only simple epithelial marker expressed by the epithelial cell layers is CK 19. The intensity of staining, however, is gradually reduced.

The results of the biochemical analysis confirmed the immunohistochemical findings. At the 10th week, CK 19 is present as a major band, but CK 8 and 18 are not detectable in gels of all ages. Their expression was identified by immunohistochemistry. In 14th week epithelia, CK 19 is still one of the major keratins. The amounts of CK 19 gradually declined up to the 23rd week. In the developing epidermis, the periderm disappeared at the 23rd week and the simple epithelial cytokeratins are no longer detectable in the follicullar epidermis (Dale *et al.*, 1985; Dale and Holbrook, 1987). In contrast, the lingual epithelium continued to express CK 19.

Cytokeratins 4 and 13 are expressed from the 10th to the 23rd week by both ventral and dorsal lingual epithelia. However, the external surfaces of some lingual papillae no longer contained CK 13 after week 19. This was more marked in the 23rd week and was

Fig. 17. Two-dimensional gel of the ventral tongue surface of a 23-week-old fetus. *Cytokeratins 4, 5, 6, 13, 14, and 16 are present with traces of 15 and 17. The added reference molecules are A= actin, Mr 42 kD, pHi 5.4; P= phosphoglycerokinase, Mr 68 kD, pHi 7.4; B= Bovin serum albumin, Mr 68 kD, pHi 6.35.*

Fig. 18. Two-dimensional gel of dorsal tongue surface of a 23-week-old fetus. *Note the presence of CK 4, 5, 6, 13, 14, and 16, vimentin (v) and desmin (d).*

Fig. 19. Two-dimensional gel of an extract of ventral tongue surface from a 19-week-old fetus. *Note the presence of major bands of cytokeratins 5 and 13 in addition to CK 4, 15 and 19. The minor bands are CK 6, 14, and 16. Vimentin is marked (v), desmin (d), and the other bands may be lamins (la).*

Fig. 20. Two-dimensional gel of an extract of dorsal tongue surface from a 19-week-old fetus. *Cytokeratins 4, 5, 6, 13, 14, 15, 16, 17 and 19 are present. Vimentin (v), desmin (d), and lamins (la).*

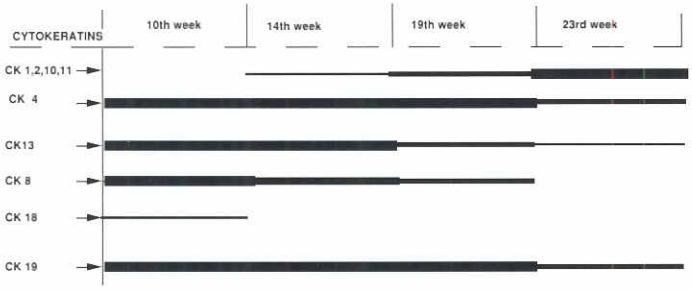
Fig. 21. Two-dimensional gel of ventral tongue surface at 14 weeks. *The major bands observed are CK 4, 5, 6, 13, 16, 17 and 19. We can also find traces of CK 14 and 15. Vimentin (v), desmin (d), and lamins (la).*

Fig. 22. Two-dimensional gel of dorsal tongue surface at 14 weeks. *The major CK found are CK 4, 5, 6, 13, 16, 17 and 19, and the minor bands are CK 14 and 15. Vimentin (v), desmin (d), and lamins (la).*

Fig. 23. Two-dimensional gel of fetal tongue, dorsal and ventral surfaces, at 10 weeks. *Cytokeratins 5, 13, and 19 formed major bands and CK 16 formed a minor band. Vimentin (v), desmin (d), and lamins (la).*

TABLE III

DYNAMIC REPRESENTATION OF COMBINED BIOCHEMICAL AND IMMUNOHISTOCHEMICAL DATA IN TOP OF FUNGIFORM AND TIP OF FILIFORM PAPILLAE OF FETAL HUMAN TONGUE, FROM 10 TO 23 WEEKS



accompanied by similar reduction in CK 4. The electrophoretic patterns showed that CK13 is expressed as early as the 10th week, while CK 4 was identified in gels of the 14th week. Similarly, CK13 was detected in gels of embryonic epidermis (8th week) (Moll *et al.*, 1982b), whereas CK 4 was expressed in the 15th week (Van Muijen, 1987). These two polypeptides disappear from the fetal epidermis by the 20th week (Van Muijen, 1987), which is not the case in the lingual epithelium during intra-uterine and postnatal life.

Terminal differentiation cytokeratins (CK 1, 2 and 10-11) are absent during the stage of epithelial stratification (10th week). They can first be detected immunohistochemically at the 14th week in some cells on the external surface of some lingual papillae. At the 19th week, the number of positive cells and the number of papillae containing these cells increased markedly up to the 23rd week, where the external surfaces of all papillae are positive. This reaction was clearly limited to the tops of the fungiform and the tips of the filiform papillae. It is interesting to note that these positive areas for terminal differentiation markers were negative for CK 4 and 13, which are considered to be markers for non-keratinizing stratified squamous epithelia. However, the tops of very few papillae did react positively for both types of marker.

These results, plus our histological findings clearly indicate that terminal differentiation markers are expressed several weeks earlier than the formation of a well-distinguished keratinized layer.

The expression of terminal differentiation markers in the 14th-19th week period can be considered as a predictive marker of keratinization. This also happens in the epidermis, when the epidermal cells express terminal differentiation keratins from the 14th week and histologic keratinization starts in the 23rd week (Moll *et al.*, 1982b; Dale *et al.*, 1985; Dale and Holbrook, 1987). In the epidermis, the cells containing terminal differentiation markers are covered by the periderm, which expresses CK 4, 8,13, 18 and 19 (Van Muijen, 1987). In the lingual epithelium it appears that the tips of the lingual papillae penetrate the periderm, since the superficial cells express the terminal differentiation markers, not the periderm markers.

The present study did not include the immunohistochemical distribution of CK 5, 6, 14, 15, 16 and 17, but these keratins were detected on the two-dimensional gels. The concentration of CK 5 did not seem to change during development. Cytokeratin 6 was not detected at the 10th week, but became one of the major components in the gels from the 14th week. In the ventral epithelium, CK 6 band became fainter from the 19th week, but the band for this cytokeratin remained strong in the dorsal epithelium gels. CK 6 was the most prominent protein, together with CK 13, in all lingual epithelia of 23-week-old fetuses. In contrast to CK 6, which appeared in the 14th week, CK 16 was detected as early as the 10th week and was present in gels of all ages in small quantities, regardless of site. CK 15 is present as traces from the 14th week to the 19th week in all lingual epithelia. At the 23rd week, the ventral epithelium expresses only traces of this cytokeratin, and this protein cannot be seen in gels of the dorsal surface. The expression of CK 5, 14, 15 and 17 in the developing skin (Dale *et al.*, 1985; Dale and Holbrook, 1987) is almost similar to that seen in developing tongue. CK 6 was a minor band and CK 16 was not detectable before the 39th week.

The electrophoretic profile of cytokeratins in adult human dorsal tongue surface is very similar to that of the 23-week-old fetus (CK 4, 5, 6, 13, 14, 15, 16 and 17). However, the gels of adult tongue show bands for the terminal differentiation cytokeratins, which indicates more synthesis of these markers after the 23rd week. The ventral tongue epithelium shows no further changes after the 23rd week, as the electrophoretic patterns are the same as those of the adult ventral tongue epithelium (Dhouailly *et al.*, 1989; Sawaf *et al.*, 1989a; 1990).

In intra-uterine life, the developing epithelium passes through three stages. The embryonic stage epithelium, up to 8 weeks, expresses simple epithelial cytokeratins. The following two fetal stages are the stratification stage between the 9th and 14th weeks, in which esophageal cytokeratin markers are produced, and the terminal differentiation stage, after the 14th week, in which skin-type markers of terminal differentiation are expressed. Our results confirm these steps from the 10th week onwards. This evolution in the expression of cytokeratins during development of the embryonic/fetal lingual epithelium could be significant, as pathologically affected adult epithelium, particularly tumoral epithelium and inflammatory epithelium, undergoes a dedifferentiation with reductions in terminal differentiation markers and increases in esophageal and simple epithelial markers (Bosch *et al.*, 1989; Sawaf *et al.*, 1989b; Ouhayoun *et al.*, 1990).

Materials and Methods

Specimens

All tissues were obtained from human fetuses after therapeutic abortions. Fetal tongues were obtained at ages 10, 14, 19 and 23 weeks; two at each fetal age. Care was taken not to include fetuses with any dermatological disease or having any influence on epithelial differentiation. The body of the tongue was cut into 2 pieces to separate the dorsal and lingual surfaces. Serial 6 µm-thick frozen sections were cut at -20°C, dried for one hour at room temperature, and treated for histology using hematoxylin and eosine, and Masson trichrome stainings. Other sections were used for immunohistochemical detection of cytokeratin polypeptides.

Indirect Immunofluorescence microscopy

Frozen unfixed tissues were cut on Reichert cryostat. The 5 to 10µm thick sections were then air dried for at least 30 min at room temperature and the slides were immediately used or stored at -80°C. They were then incubated with the appropriate monoclonal antibody for one hour at 37°C. After three washings (10 min each) with phosphate buffered saline (PBS), the slides were dried and the sections were incubated with goat antimouse (IgG H+L) FITC-labeled antibody for one hour at 37°C. After three new washings (10 min

each) with PBS, the slides were dried and mounted with aquamount (BDH) and coverslips. Negative controls were performed by replacing the primary antibody with PBS. The sections were then examined by Leitz fluorescence microscopy.

Antibodies

The 14 mouse monoclonal antibodies used for the detection of cytokeratin proteins (all IgG) in tissue sections are given in Table I with their references. We have selected two broad spectrum antibodies, used as broad markers of epithelial cells (KL1 and Ks1-8), and 12 antibodies specific of a single or a small subset of cytokeratin polypeptides. Some specific antibodies against cytokeratins (9,12,15,17, 20) were not available to us at the time of experiment.

Biochemistry

The cytokeratin electrophoretic profiles of dorsal and ventral epithelia from 14, 19 and 23 week fetuses were obtained. The tongues of 10-week-old fetuses were too small for dissection, so the cytokeratin profiles of both dorsal and ventral epithelia were characterized together. Cytokeratins were extracted as described by Achtstätter *et al.* (1986). Briefly, the tissue specimens were cut into 20 µm-thick sections at -20°C and homogenized in a high salt-detergent buffer (1.5 M KCl, 10 mM Tris-HCl, 150 mM Na Cl, 1% v/v Triton X-100, 5 mM EDTA, pH 7.4). The homogenate was centrifuged for 10 min at 5000 rpm, and the pellet was resuspended in a low salt buffer, stirred for 5 min at 4°C, and centrifuged again. The pellet was washed in PBS, centrifuged once more, and finally stored at -70°C.

Two-dimensional gel electrophoresis using non-equilibrium pH gradient electrophoresis (NEPHGE) in the first dimension was performed by methods described by O'Farrell *et al.* (1977), and modified by Franke *et al.* (1981). The ampholine range was pH 2-11, and the lysis buffer contained 0.25% sodium dodecyl sulfate. The second dimension, (Laemmli, 1970) separated the cytokeratin polypeptides according to their molecular weight, using 10% separating gel and 3.9% stacking gel with an acrylamide/bis ratio of 30:0.8. The gels were stained with Coomassie blue.

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