Pergamon

Oral Oncol, Eur J Cancer Vol. 32B, No. 5, pp. 352–358, 1996

Printed in Great Britain
0964-1955/96 $15.00 + 0.00

PII: S0964-1955(96)00023-1

Expression of Cytokeratins in Warthin's Tumour (Adenolymphoma) of Parotid Glands: Specific Detection of Individual Cytokeratin Types by Monoclonal Antibodies

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This study evaluated the distribution of cytokeratins detected by monoclonal antibodies directed against individual keratin proteins in normal human salivary glands and epithelial tumour cells of Warthin's tumour arising in parotid glands to determine a more precise mapping of their cellular distribution. The normal salivary ducts showed the presence of cytokeratins 7, 8, 18 and 19 in the intercalated, striated and excretory ducts, the primary keratins of stratified and simple epithelia with a profile very similar to the non-cornified epithelium of the oral mucosa. The basally located cells of salivary gland ducts other than myoepithelial cells were reactive for keratins 7 and 19 suggesting a close similarity in profile of keratin in the basal cells of the oral epithelium. In Warthin's tumour, keratins 7, 8, 18 and 19 were consistently detected in the epithelial cells of the tumour, a profile with a tendency to mimic the same in normal ductal epithelium. The distribution, however, was diverse and a heterogeneity was observed in the basal and luminal cells of Warthin's tumour which differed even in different areas of the same tumour specimen. Copyright © 1996 Elsevier Science Ltd

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INTRODUCTION

The expression of cytokeratins, the epithelial cell-specific intermediate proteins, has been widely investigated in human neoplasia, including neoplastic lesions of salivary glands. The patterns of expression of cytokeratins are of practical significance in cancer diagnosis as they are generally expressed in a tissue specific manner. Based on immunohistochemical observations, the expression of keratins is tightly linked with epithelial differentiation where the composition and patterns of expression in a normal epithelium are reflected to a large extent in their neoplastic lesions.

There are extensive overviews and reviews dealing with the regulation of expression, assembly, interactions with other cell structures and possible functions of cytokeratins [1]. Keratin filaments are assembled from heterodimer subunits composed of a type I acidic (nos. 9–20) and type II basic (nos. 1–8) cytokeratin and each is encoded by its own gene [2]. Although many keratins are present in a particular epithelial cell, the acidic and basic keratins are present in an equimolar amount. In normal epithelia, keratin pairs are expressed in a consistent manner and its numerical classification have an implication in defining its tissue distribution. Thus, pairs of K7 and K19, and K8 and K18 are present in simple epithelial cells while K5 and K15 are present in the basal cells of stratified and pseudostratified epithelia [3].

Immunohistochemical studies on expression of cytokeratins in normal and neoplastic lesions of salivary glands have been carried out mostly by using monoclonal antibodies to several groups of keratin proteins [4–9]. However, the use of monoclonal antibodies to individual keratin proteins would enable a more precise mapping of cellular distribution of cytokeratins and this would be of more importance in studying neoplastic lesions showing a heterogeneous differentiation of tumour cells such as those of salivary glands.

Adenolymphoma, also known as papillary cystadenoma lymphomatosum or Warthin's tumour of salivary glands, histopathologically show epithelial and lymphoid tumour components where the former contains luminal eosinophilic columnar cells and basally located flat or cuboidal basal cells. The present study describes the profile of distribution of cytokeratins using monoclonal antibodies to individual cytokeratin proteins, in an attempt to localise individual

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Received for publication 23 Apr. 1996.

Table 1.

Monoclonal antibody	Dilution	Source	References
K4	1:300	Sigma Immunochemicals, U.S.A.	[10]
K7	1:200	Sigma Immunochemicals, U.S.A.	[11]
K8	1:200	Sigma Immunochemicals, U.S.A.	[12]
K10	1:100	Dakopatts, Denmark	[13]
K13	1:100	Sigma Immunochemicals, U.S.A	[14]
K17	1:20	Dakopatts, Denmark	[15]
K18	1:800	Sigma Immunochemicals, U.S.A	[14]
K19	1:50	Sigma Immunochemicals, U.S.A	[13]
KL1	1:50	Immunotek, France	[8, 9]
K8.12	1:50	Biomakor, Israel	[8, 9]

keratin proteins, in the epithelial tumour component of salivary gland adenolymphoma.

MATERIALS AND METHODS

A total of 23 cases of adenolymphoma of salivary glands were evaluated for expression of cytokeratins using monoclonal antibodies to individual keratin proteins. The tumour specimens obtained from surgery were fixed in 10% neutral buffered formalin for 12 h and embedded in paraffin. Specimens of normal salivary glands (submandibular and parotid) obtained from radical neck dissections or parotidectomy fixed in Bouins solution (12 h), 10% formalin

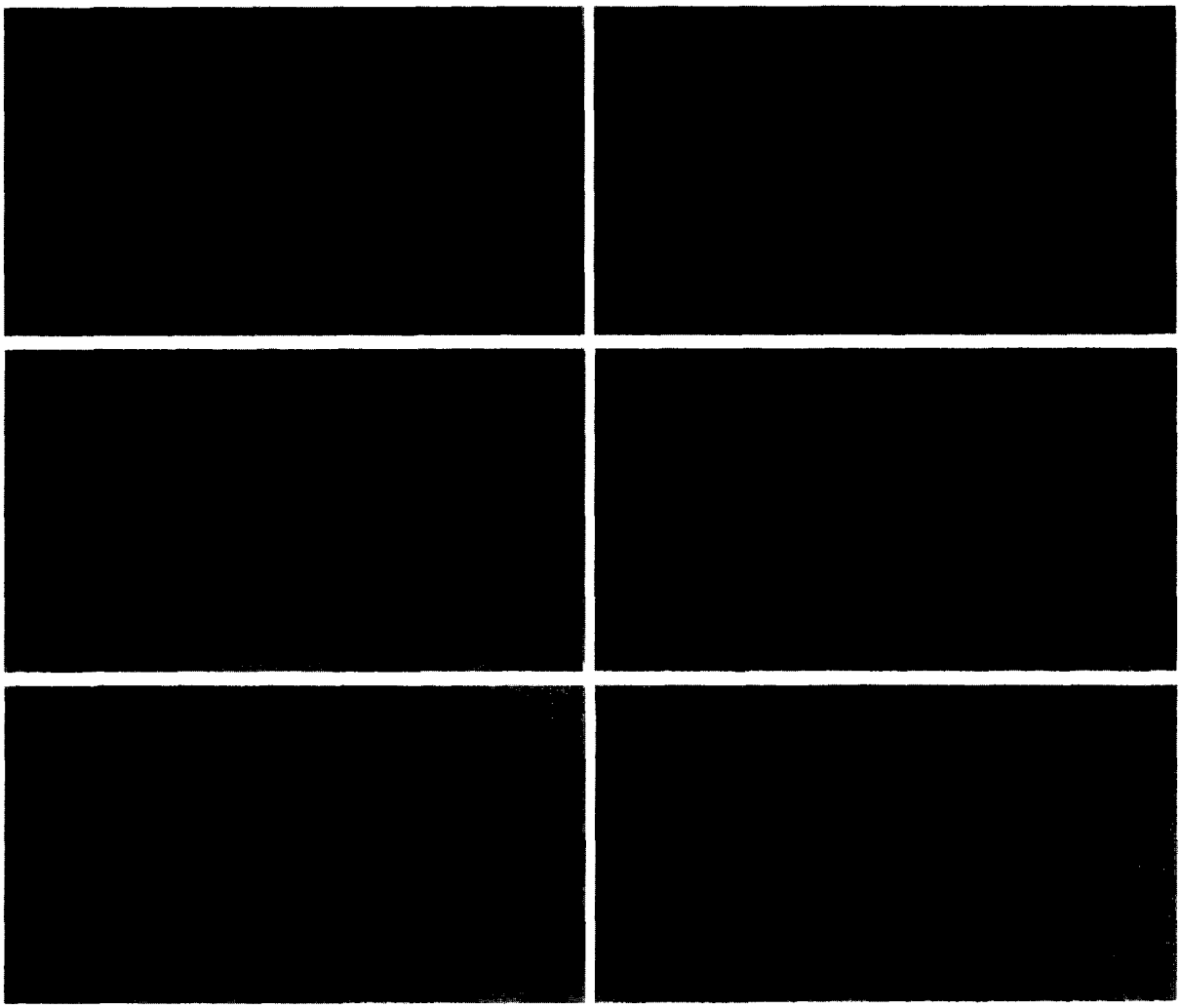

Fig. 1A-F.

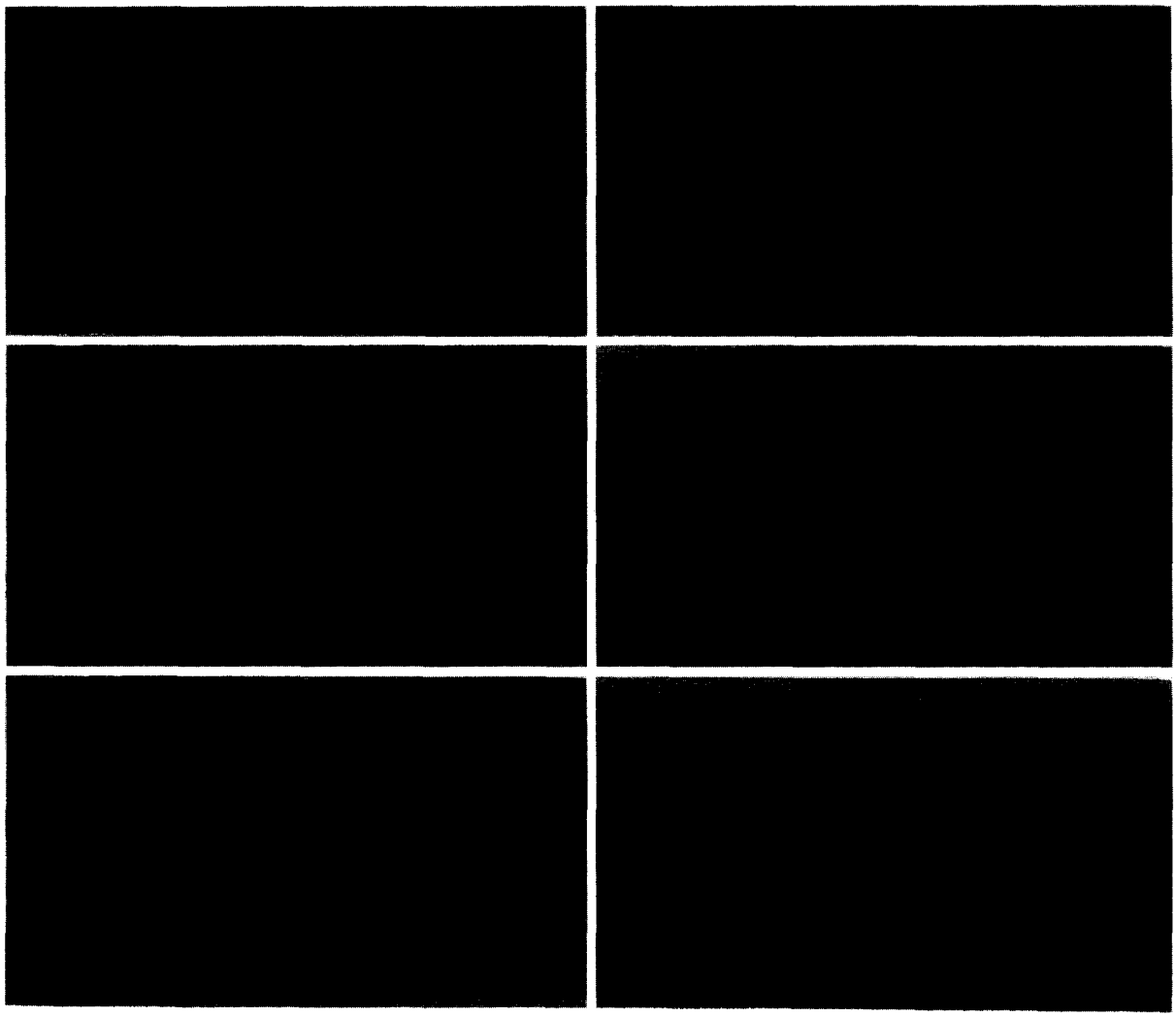

Fig. 1. Immunostaining of keratins in normal submandibular gland (×100): (A) K7, formalin fixed sections. Intercalated and striated duct cells show positive immunostaining. (B) K7, Carnoy fixed solution. Intercalated and striated ducts show an intense immunostaining. (C)–(F) K8. (C) formalin fixed section, striated duct cells show positive staining. However, ductal basal cells are devoid of staining; (D) Carnoy fixed section. Intercalated duct shows an intense reaction. (E) and (F) Bouin fixed solution. Striated duct (E) and excretory duct (F) show an intense staining. The acini show staining of the cell membrane while ductal basal cells in the ductal segment are negative for K8. (G) K13 in formalin fixed solution. A weak staining is seen in the ductal epithelium. (H)–(J) K19. (H) Carnoy fixed section. K19 is seen in the ductal epithelium, particularly in the luminal surface of the cells and the basal cells are negative. (I) Bouin fixed section. Irregular staining of K19 is found in the ductal segment, particularly the luminal cells. (J) Bouin fixed section. Excretory duct epithelium shows prominent K19 reaction at the luminal surface. (K) and (L) Monoclonal antibody K8.12, (K) Carnoy fixed section. Striated and excretory ducts show positive staining for K8.12. The ductal basal cells, in particular, express keratin detected by MoAb K8.12. (L) Carnoy fixed solution, large excretory duct epithelium shows marked staining for K8.12 in basal cells and the luminal columnar cells are also stained.

(12 h) or Carnoy solution and embedded in paraffin were employed for normal controls. Four micrometre sections were evaluated for histopathological features using HE staining and immunohistochemical detection of cytokeratins using a streptavidin biotin immunoperoxidase kit obtained from Dakopatts, Denmark. Briefly, the sections were deparaffinised and treated with a 0.01% solution of protease for 5 min at room temperature. The sections were then treated with methanol containing 0.3% H_2O_2 for 20 min. The primary antibodies were commercially available and are shown in Table 1. Subsequent steps of immunohistochemical methods have been described elsewhere [16].

RESULTS

Normal salivary glands

The immunohistochemical localisation of keratins in the tissue specimens was negative or very weak for K4, 10, 13, 17 and 20 (Table 2). K7 was present in the intercalated duct cells and luminal cells of excretory ducts (Fig. 1A, B). K8 was seen in the intercalated duct and acinar cells. However, the basal cells of striated and excretory ducts were not reactive (Fig. 1C–F). K13 was weak in the striated and excretory ducts (Fig. 1G). Reaction products for K18 and K19 were most prominent in the intercalated, striated and excretory duct cells (Fig. 1H), while those specimens

Table 2. Immunostaining of individual keratins by monoclonal antibodies in normal human parotid and submandibular glands

Keratin	ME	A	IC	SD	EDL	EDB
K4	0	0	0	0	0	0
K7	0	0	+ – ++	+	+ – ++	+/–
K8	0	+ – ++ + – ++	++	++	0	0
K10	0	0	0	0	0	0
K13	0	0	0	0 – +	0 – +	0
K17	0 – +/–	0	0	0	0	0
K18	0	0	+	+ – ++	++	0
K19	0	0	+/–	+/– – +	+ – ++	0
K20	0	0	0	0	0	0
KL1	0 – ++	+/– –	+ + – ++	+ – ++	+ ++	+ ++
K8.12 [13, 15, 16]	0	0	+ – ++	+ – ++	+ – ++	+ ++

ME, myoepithelial cells; a, acinar cells; IC, intercalated duct cells; SD, striated duct cells; EDL, excretory duct luminal cells; EDB, excretory duct basal cells. 0, negative; +/–, trace; +, weak; ++, moderate; +++, strong.

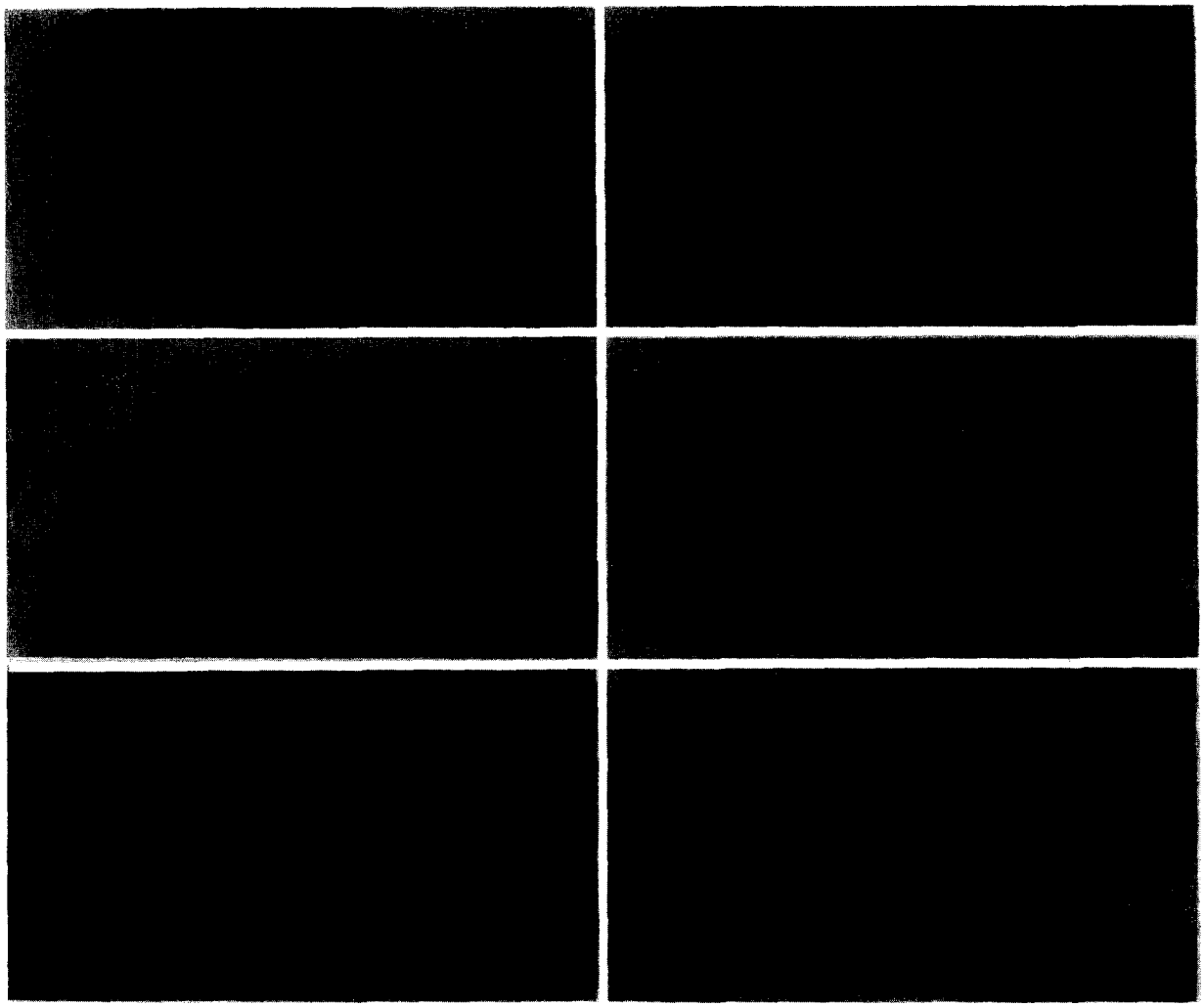

Fig. 2. (A)–(C) Adenolymphoma. Serial sections from formalin fixed sections. ×100. (A) K7. Striated high columnar epithelium of the tumour shows an irregular expression of K7 keratin. (B) K8. High columnar tumour epithelium shows an irregular staining ranging from negative to strong. (C) Monoclonal K8.12 shows an intense staining of the basal tumour cells. (D)–(F). Adenolymphoma. Serial sections of formalin fixed section. ×100. (D) K7. High columnar cells show a positive staining while basal tumour cells are negative. (E) K8 staining. The distribution is very similar to that of K7. (F) Monoclonal K8.12 shows an intense staining of the basal and the luminal tumour cells.

Table 3. Immunostaining of keratin using monoclonal antibodies in epithelial tumour cells of adenolymphoma

Keratin	Basal cells	Columnar cells	Luminal surface
K4	− (23)	− (23)	− (23)
K7	+/− (8)	+ − ++ (19)	+ − ++ (9)
K8	+/− (15)	+/− − + (17)	+ + (14)
K10	− (23)	− (23)	− (23)
K13	− (23)	+/− (1)	− (23)
K17	− (23)	− (23)	− (23)
K18	+/− − +/− (4)	+/− (10)	− (23)
K19	+/− (6)	+ (15)	+ − ++ (10)
K20	− (23)	− (23)	− (23)
KL1	++ (23)	++ (23)	+ + (19)
K8.12	+ + − + ++ (20)	− −+ (20)	+ (11)

0, negative; +/−, trace; +, weak; ++, moderate; +++, strong (figures in parentheses indicate the number of cases).

fixed in Bouins showed relatively low reaction in the striated and excretory duct epithelium (Fig. 1I, J). Staining intensity on the luminal side of luminal cells of the excretory duct was intense as compared to basal cells for K19.

Monoclonal antibody KL1 labelled all the ductal cells with varying intensity of reaction. However, it was rarely reactive to myoepithelium and acinar cells. Monoclonal K8.12, on the other hand, was prominent in the ductal basal cells and in luminal epithelial cells of the ductal system (Fig. 1K, L).

Adenolymphoma (Table 3)

The monoclonal antibodies showing no staining of the normal salivary gland epithelium were unreactive in the adenolymphoma epithelial tumour cells. K4, K10, K13, K17 and K20 were negative in all cases. However, in some areas of the tumour specimen a weak or trace immunoreactivity was observed. K7 and K8 were present in tall columnar cells approximating the lumens of large cystic spaces where the intensity of staining for K8 was more heterogeneous, ranging from unreactive to strongly reactive cells in different areas of the same specimen (Fig. 2A, B). The inner or the basal layer of cuboidal or polygonal cells predominantly showed immunoreactivity with monoclonal K8.12 (Fig. 2C). Epithelial cells approximating smaller cystic spaces also had K7 and K8 in the tall columnar luminal cells and reaction products for K8.12 in basal cells (Fig. 2A–C).

The profiles of immunoreactivity for K18 and K19 were very similar to that for K8 in such a way that only the luminal surface of luminal cells may show an intense immunostaining, or all the luminal cells show immunostaining (Fig. 3A–D). In numerous instances the luminal or tall columnar cells showed a heterogeneous intensity of reaction for K18 and K19 where few cells were intensely labelled or others were negative or with varying intensity of staining (Fig. 3E, F).

The monoclonal anticytokeratin KL1 labelled all the tumour epithelial cells while K8.12 had a characteristic immunostaining of the basal tumour cells, although numerous luminal cells of the ducts were also stained.

DISCUSSION

This study aimed to determine the distribution of keratins detected by monoclonal antibodies directed against individual keratin proteins in the epithelial tumour cells of adenolymphoma which consists of two types of histologically distinct cells, the basal and apical columnar cells, and compare the results with those in the ductal system of normal salivary glands. In previous studies, Caselitz *et al.* [17], Born *et al.* [18], Orito *et al.* [19] and Tanaka *et al.* [20] have demonstrated the profile of keratin distribution in adenolymphoma by using monoclonal antibodies that detect keratin pairs or several types of keratin. The basal cells in adenolymphoma has been found to be immunoreactive with monoclonal K8.58 (nos 13 and 16) and K8.12 (nos 13, 15, 16) [17, 19]. Basal cells of normal salivary glands have been found to be reactive with monoclonal K8.58 [18].

The presence of keratin pairs with acidic and basic keratins has been extensively studied in normal and neoplastic tissues. In normal oral epithelium, K5 and K14 are present in basal and suprabasal cells of keratinised epithelium of masticatory mucosa and the presence of K1 and K10 has been regarded as a marker of a differentiation-specific pair which is present in the cornified stratified layer of epithelia. The K8 and K18 pair are primary keratins of stratified and simple epithelia. Furthermore, K4 and K13 are present in non-keratinised epithelium whereas K4, K13, K6 and K16 are present in suprabasal cells and are regarded as markers of epithelial differentiation [3]. Profiles of distribution of keratin in normal oral mucosa and specific alterations in their expression in numerous hyperproliferative or premalignant and malignant lesions have been widely investigated [21, 22]. However, attempts to determine such a profile in normal salivary glands and their various benign and malignant neoplasms have been unyielding. It may possibly be due to a complex phenomenon of development of the salivary glands, the most heterogeneous tumour subtypes and differentiation of tumour cells in salivary neoplasms [23].

In general, for descriptive purposes, histologically the principal cell types and their organisation in salivary glands may be regarded as having two major cell types, luminal and non-luminal/basal. Therefore, the salivary unit, extending from the ductal system to the acini, may be described as consisting of the duct luminal cells and acini the 'luminal', which at all levels are associated with ductal basal and/or myoepithelial cells, the non-luminal cell type [23]. A population of reserve stem cells believed to be associated with the excretory ducts or intercalated ducts have been implicated in the genesis of salivary tumours [24]. However, direct evidence is lacking. Since salivary glands initially

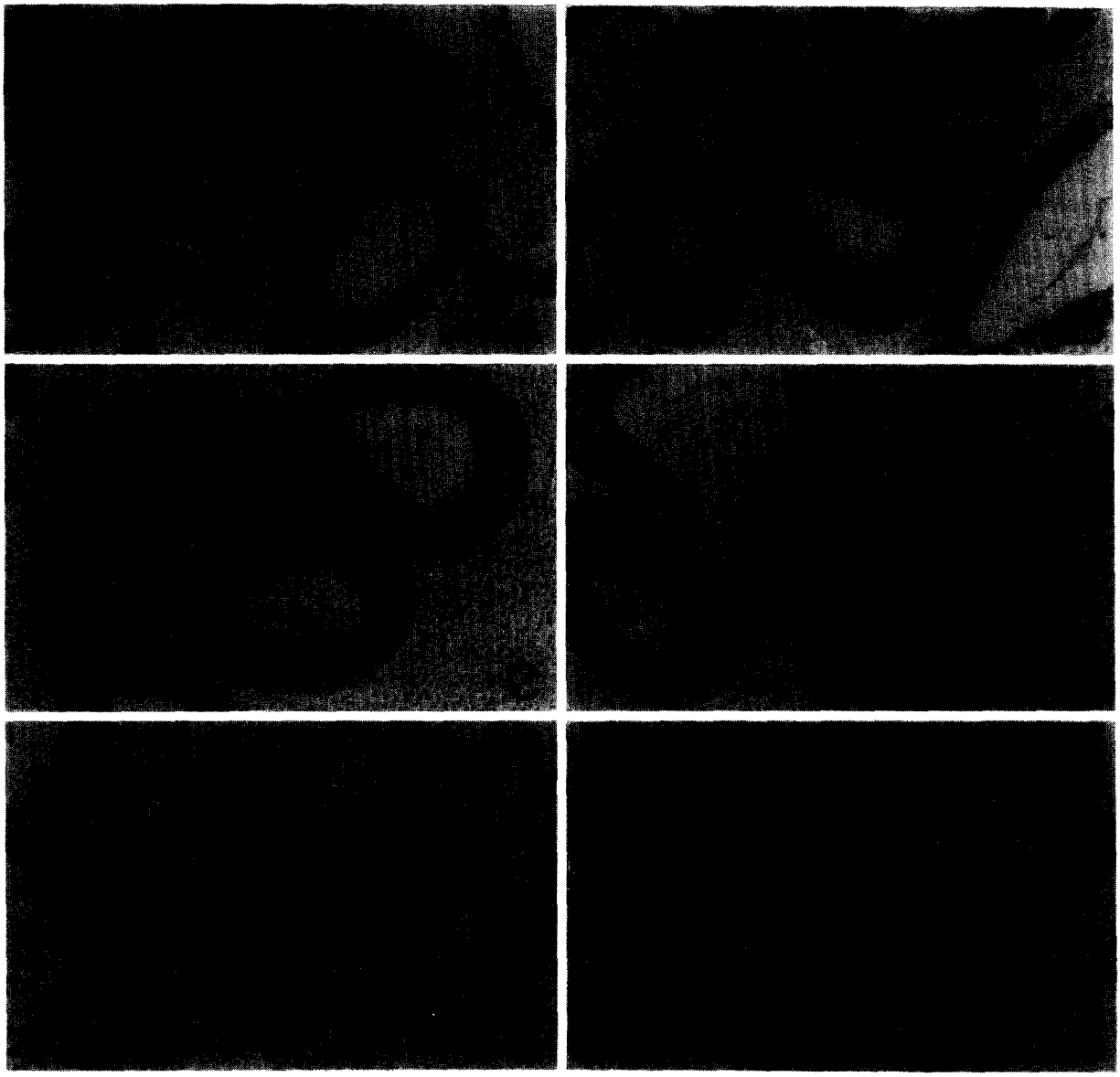

Fig. 3. Keratin profile in well proliferating tumour cells of adenolymphoma. ×100. (A) K8, the luminal surface of tumour epithelium shows an intense immunostaining, the basal tumour cells are negative. (B) K18. Luminal aspect of the luminal cells shows an intense staining. (C) K8. All luminal cells show an intense staining while basal cells are devoid of K8. (D) K19. Luminal side of columnar cells stains positively. However, a few luminal cells remain unstained. (E) K19, Columnar cells show a heterogeneous intensity of reaction for K19, few cells are intensely labelled while others are negative. (F) K18. Most of the tumour cells show an intense immunostaining and staining on the luminal surface is more prominent.

develop from the invagination of oral epithelium which subsequently is followed by a complex morphogenesis, in the present study, we have attempted to determine and compare the distribution of keratin in normal salivary gland ducts and oral epithelium. The results of the present study in normal salivary ducts showed the presence of cytokeratins 7, 8, 18 and 19 in the intercalated, striated and excretory ducts, which are primary keratins of stratified and simple epithelia with a profile very similar to the non-cornified epithelium of the oral mucosa. In particular, the basally located cells of salivary gland ducts other than myoepithelial cells were positive for keratin 7 and 19 but not for 8 and 18, suggesting a close similarity in profile of keratin in normal salivary ducts with the basal cells of oral epithelium.

Studies on the epithelial tumour component of adenolymphoma at the immunohistochemical and ultrastructural level have shown that they resemble the ductal basal cells of normal salivary glands more and there is no participation of myoepithelial cells [20]. However, a proportion of basal cells in the tumour have been suggested to be capable of differentiating as myoepithelial-like cells in Warthin's tumour [25] and the participation of myoepithelial cells in salivary tumours is one of the most controversial topics. Therefore, if the epithelial tumour cell component of adenolymphoma is to be taken as a model for a specific pattern of differentiation of ductal cells, it does seem logical to conclude, on the basis of detection of individual keratin types in the present study. Although there exists a heterogeneity

of distribution of individual keratins in the luminal and non-luminal or basal cells of adenolymphoma, in the majority of instances they tend to mimic a profile similar to the normal ductal epithelium. Thus, K4, K10, K13, K17 and K20, virtually not detected in normal salivary ducts, were negative in the epithelial tumour cells of adenolymphoma. On the other hand, K7 and K8, detected in the basal tumour cells of adenolymphoma in one third to one quarter of the cases were also present in the luminal cells in more than three quarters of the cases. Similarly K17 and K18 in basal cells of nearly half to two thirds of the cases also had similar distribution in the luminal or columnar tumour cells. Most obviously, in the same tumour specimen, there were cells showing an intense immunoreactivity as well as those without any reaction products.

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