Virchows Arch A (1987) 411:583–589

Virchows Archiv A

Cytokeratin expression in normal salivary glands and in cystadenolymphomas demonstrated by monoclonal antibodies against selective cytokeratin polypeptides

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Summary. The distribution of selective cytokeratin polypeptides, vimentin, and glial fibrillary protein (GFP) in 5 human cystadenolymphomas of the parotid gland was compared with normal human parotid ($n=5$) and submandibular ($n=4$) glands using a panel of monoclonal antibodies against diverse and selective cytokeratin polypeptides, vimentin and glial fibrillary protein (GFP). A biotin-streptavidin method was used on cryostat sections. The immunocytochemical finding of identical cytokeratin polypeptides Nos. 7, 8, 18 and 19 and basal cells selectively labeled by the monoclonal antibody KS 8.58, in both the epithelial part of the cystadenolymphomas and in the duct epithelium of the parotid gland, confirms the hypothesis that the epithelial compartment of cystadenolymphomas is derived from the duct system. The triple expression of cytokeratin, vimentin and GFP in myoepithelial cells of the parotid gland is discussed.

Key words: Cystadenolymphoma – Salivary gland – Histogenesis – Cytokeratin – Vimentin – Glial fibrillary protein (GFP) – Co-expression

Introduction

Cystadenolymphomas comprise about 15% of all salivary gland tumours and more than 70% of monomorphic adenomas (Seifert et al. 1984). Thompson and Bryant (1950) were the first to formulate the theory of the development of cystadenolymphomas from neoplastic duct proliferations in salivary glands.

In order to confirm the putative histogenesis of cystadenolymphomas from the salivary duct system on a molecular basis, cytokeratins were used as specific structural epithelial markers. The possibilities and limitations of immunocytochemical methods with regard to the pathology of salivary glands and their tumours have been discussed repeatedly (Caselitz et al. 1981; 1982a, b; 1984; Krepler et al. 1982; Seifert 1984; 1985).

Cytokeratins (CK) represent the epithelial class of intermediate-sized filaments which are part of the cytoskeleton (Osborn et al. 1982). Up to date, 19 diverse CK polypeptides have been identified from cytoskeleton preparations of human tissues by the use of two-dimensional gelelectrophoresis (Moll et al. 1982). It is of great importance that (a) the distribution of individual CK polypeptides depends on the differentiation of epithelial cells and tissue, and (b) CK, like the other classes of IF, are expressed in tumours (for recent review, see Moll 1986).

The aim of the present study was to characterize and define the diverse cell types of the human salivary glands and the epithelial compartment of cystadenolymphomas on a molecular basis, using a panel of monoclonal antibodies against selective CK polypeptides, vimentin and GFP. It was our intention to relate the epithelial part of cystadenolymphomas to a compartment of the secretory unit within the salivary gland and thus to contribute to the knowledge of histogenesis of cystadenolymphomas.

Materials and methods

Biopsy specimens of 5 cystadenolymphomas, 5 parotid glands and 4 submandibular glands were cut in small pieces and snap frozen in liquid nitrogen immediately after surgical removal (Universitäts-Hals-Nasen-Ohrenklinik Gießen and Heidelberg, FRG). 5–6 μm cryostat sections were performed using a Frigocut 2800 E (Fa. Reichert-Jung, Nußloch, FRG). Air dried slides were fixed in acetone for 5 min at −20° C and stored at −70° C

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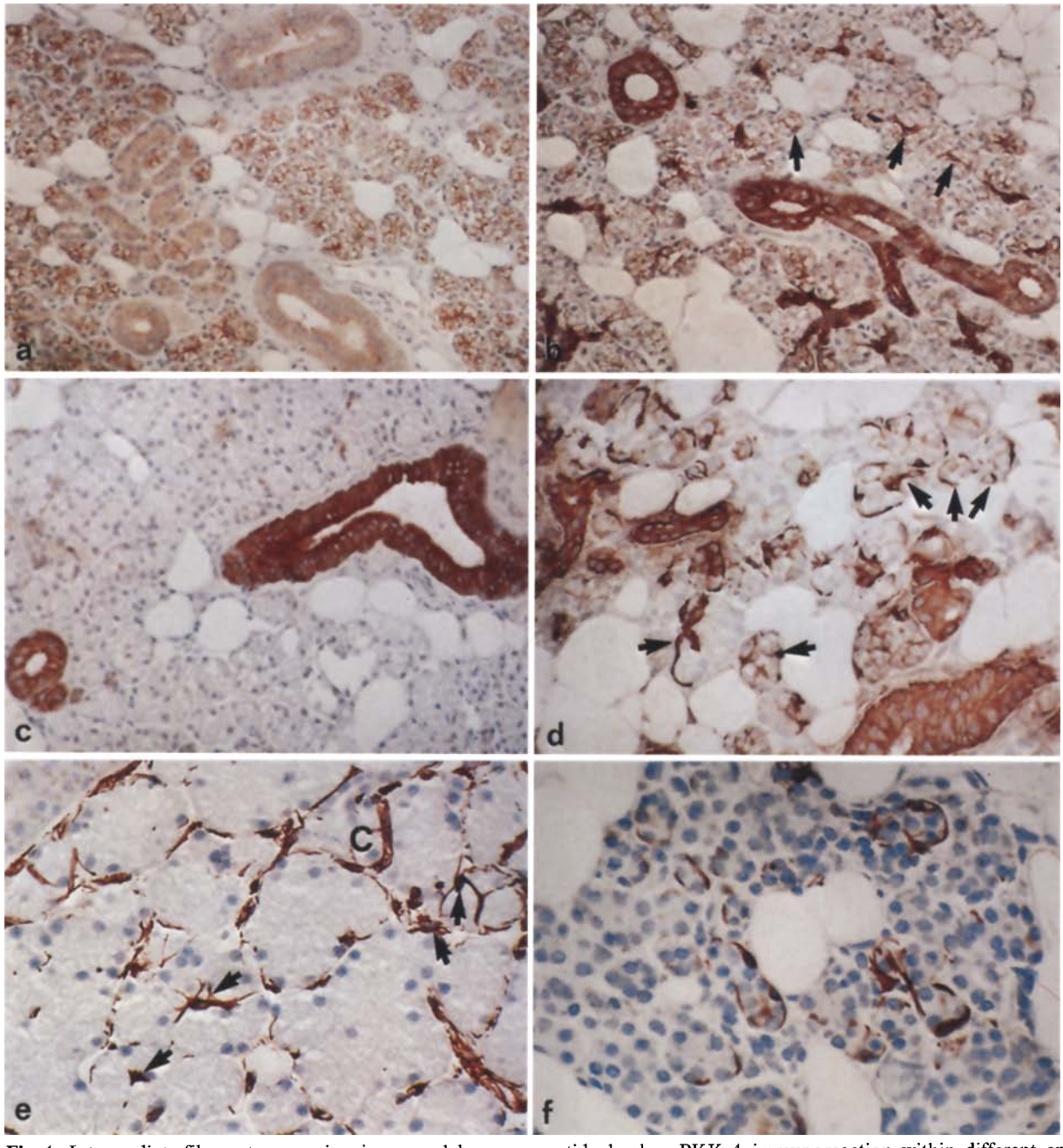

Fig. 1. Intermediate-filament expression in normal human parotid gland. **a** PKK 1 immunoreaction within different epithelial cell types; **b** antibody to cytokeratin 7 stains the ductal epithelium strongly and acinar cells as well as myoepithelial cells (*arrows*); **c** antibody to cytokeratin 8 reacts with striated duct (*strong*) and intercalated duct (*weak*) epithelial cells. **a–c** Original magnification: 10×. **d** Antibody to CK 19 (AE 53-B/A2) reacts strongly with duct epithelium and with myoepithelial cells of the acini (*arrows*); **e** Vimentin intermediate filaments are expressed in myoepithelial cells (*arrows*) and the glandular stroma; **f** GFP immunoreaction is restricted to myoepithelial cells. **d–f** Original magnification: 25×. **a–f** Biotin-streptavidin, AEC; haematoxylin counterstain

until use. For immunocytochemistry, a biotin-streptavidin method (Hsu et al. 1981) was employed using biotinylated sheep anti-mouse immunoglobulins (1:50, Lot No. 48) and streptavidin-biotinylated horseradish peroxidase (1:100, Lot No. 53; Amersham-Buchler, Braunschweig, FRG). Peroxidase activity was visualized by 3-amino-9-ethylcarbazole (AEC, Sigma, Munich, FRG) according to Graham et al. (1965). Nuclear counterstaining was performed by Mayer's haematoxylin. The slides were mounted in glycerol gelatine.

The following monoclonal antibodies against intermediate filaments were used: against broad reacting monoclonal cytokeratin antibodies we used: – AE 1–AE 3 (Hybritech, San Diego, California, USA; recognizing 50 KD and 56.5 KD cytokeratin polypeptides (AE 1) as well as 58 KD and 65–67 KD

Table 1. Intermediate filament protein distribution in normal salivary gland

	Cytokeratins					Vim	GFP
Cytokeratin polypeptides	7	18	8	19	13,16		
Monoclonal antibodies (clones)	CK 7	CK 2	LE 41	AE 53-B/A2	KS 8.58		
Acinus, serous	++	+++	(+)	(+)	−	−	−
mucous	++	+++	−	(+)	−	−	−
Duct epithelium	+++	++	+++	+++	−	−	−
Basal cell	−/+	−/+	−/+	+	+++	−	−
Myoepithelial cell	+/++	?	−	+++	−	+	+
Stroma	−	−	−	−	−	+	−

cytokeratin polypeptides (AE 3); Woodcock-Mitchell et al. 1982); – KL 1 (Dianova, Hamburg, FRG; this reacts with acidic keratin polypeptides of 56 KD; Viac et al. 1983); PKK 1 (Lab. Systems, Helsinki, Finland; this reacts with all cytokeratins of HeLa cells, i.e. 44, 46, 52 and 54 KD cytokeratin polypeptides; Holthöfer et al. 1983); they were used at 1:20. The monoclonal antibodies against selective cytokeratin polypeptides used were: clone K 6 (CK 1, 2; Osborn et al. 1985), clone CK 7 (CK 7; Tölle et al. 1985), clone LE 41 (CK 8; Lane 1982), clone LE 65 (CK 18; Lane 1982), clone LP 2K (CK 19; Lane et al. 1985), 1:5; Amersham-Buchler, Braunschweig, FRG; clone CK 2 (CK 18; Boehringer, Mannheim, FRG; Debus et al. 1982); Keratin RKSE 60 (CK 10; Eurodiagnostics, Apeldoorn, Netherlands; Ramaekers et al. 1983), 1:10; AE 53-B/A2 (CK 19; Dr. Kasper, personal communication) was a kind gift of Drs. Karsten and Kasper, Berlin/Görlitz, German Democratic Republic; 6B10 (CK 4; van Muijen et al. 1986) was generously given by T. Achtstätter and W.W. Franke, German Cancer Research Center, Heidelberg, FRG; KS 8.58 (CK 13, 16) was generated and characterized as previously described (Geiger et al. 1987); as monoclonal antibodies against vimentin we used clone V9 (Osborn et al. 1984) and against glial fibrillary protein we used clone G-A-5 (Debus et al. 1983). Both were purchased from Boehringer, Mannheim, FRG, and diluted 1:10.

Primary antibodies were incubated for 1 h, and biotinylated secondary antibodies and streptavidin-peroxidase-complex for 30 min, respectively, in a moist chamber at room temperature.

Controls were performed by omitting the first antibodies and always gave negative results.

Results

Non-neoplastic parotid and submandibular gland

AE 1–AE 3, KL 1 and PKK 1 which are monoclonal antibodies against diverse cytokeratin polypeptides, reacted with all epithelial cell types of the secretory unit in both the parotid and submandibular gland including myoepithelial and basal cells (Fig. 1a).

Immunoreactivity of monoclonal antibodies against the selective cytokeratin polypeptides 7, 8, 18 and 19 showed a considerable variability from gland to gland. It is also noteworth that two monoclonal antibodies directed against identical CK polypeptides (for example anti-CK 18) varied in their cellular reactivity. With these restrictions in mind the results can be summarized as follows: Anti-CK 7 and anti-CK 18 gave a strong reaction both in the mucous and serous acinar cells as well as the ductal cells (Fig. 1b). In contrast, anti-CK 8 and anti-CK 19 reacted very strongly in all parts of the duct system and gave a weak or negative reaction within the acinar cells of both the submandibular and parotid gland (Fig. 1c, d). The immunoreactivity in myoepithelial and basal cells could not always be interpreted when duct system or acini were strongly stained. Using selective CK-polypeptide specific antibodies, myoepithelial cells unequivocally expressed CK 7 and 19 (Fig. 1b, d). In contrast, reaction of cytokeratin polypeptide No. 8 as determined by the monoclonal antibody LE41 was negative. As serous and mucous acinar cells were strongly labeled by cytokeratin 18, it was not possible to decide whether myoepithelial cells express this cytokeratin polypeptide, too (Table 1).

Basal cells were definitely positive with anti-CK 19, variably stained with anti-CK 18, and were probably negative with anti-CK 7 and anti-CK 8. Single cells or rarely clusters of epithelial cells expressed CK 1+2 (CK 6), CK 10 (RKSE 60), and CK 4 (6 B 10). The monoclonal antibody KS 8.58 selectively labelled the randomly distributed basal cells in all parts of the duct systems (Fig. 3a). Vimentin, the mesenchymal type of IF, was positive within the stroma of salivary glands, lymphoid cells and blood vessel walls. It is especially noteworthy, that myoepithelial cells of the acini showed a coexpression of cytokeratins and vimentin (Fig. 1e) and some cells were in addition GFP-positive (Fig. 1f). The GFP reaction in myoepithelial cells was restricted to the parotid gland. Our

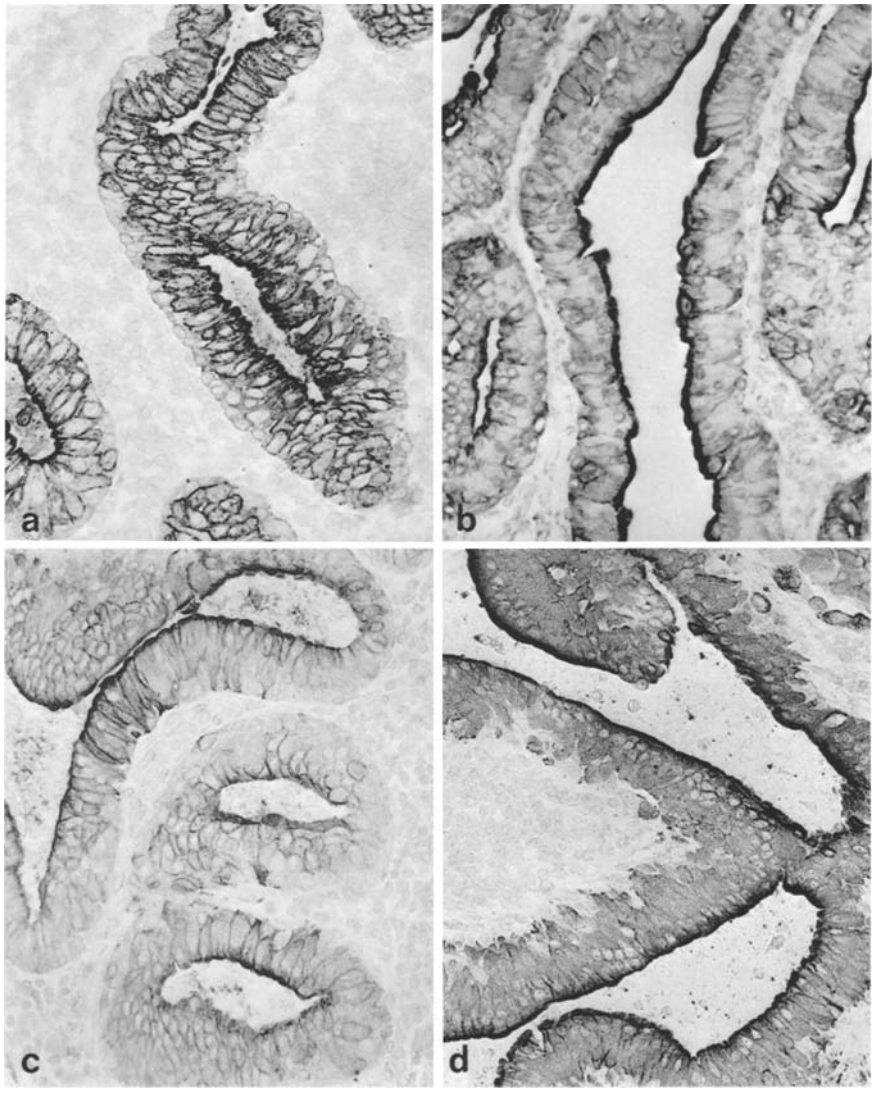

Fig. 2. Cytokeratin distribution in cystadenolymphoma. Broad reacting cytokeratin antibodies such as PKK 1 (**a**) and KL 1 (**b**) as well as selective cytokeratin polypeptide antibodies against CK 7 (**c**) and CK 8 (**d**) label all epithelial cells. Biotin-streptavidin; AEC; haematoxylin counterstain. Original magnification: 25×

results in normal human salivary glands are quoted in Table 1 and schematically illustrated in Fig. 4.

Cystadenolymphomas

The oncocytic differentiated epithelium in our 5 cases of cystadenolymphomas exhibited a strong immunoreaction with broad-reacting monoclonal cytokeratin antibodies (AE 1-AE 3, KL 1, PKK 1; Fig. 2a, b) as well as with monoclonal antibodies against cytokeratins 7, 8, 18 and 19 (Figs. 2c, d and 3c). The reaction, however, was not uniform and varied in intensity from field to field or cell to cell. As in non-neoplastic salivary glands, KS 8.58 strongly labeled the numerous basal cells (Fig. 3b). Antibodies against CK 1+2 (K 6), CK 10 (RKSE 60) and CK 4 (6B10) were positive in single cells and sometimes in cell clusters (data not shown). The lymphoid stroma of cystadenolymphomas was strongly vimentin-positive as were numerous interepithelial lymphocytes (Fig. 3d) and vessel walls. The epithelial compartment was completely negative. In cystadenolymphomas, GFP immunoreactivity was not found.

Discussion

Demonstration of cytokeratins, vimentin and GFP by immunomorphological methods allows the individual characterization of cell types within the secretory unit of human salivary glands (Table 1; Fig. 4).

While broadly-reacting cytokeratin antibodies such as AE 1 and AE 3, KL 1 and PKK 1 label the whole complement of epithelial cell types in salivary glands, selective monoclonal cytokeratins

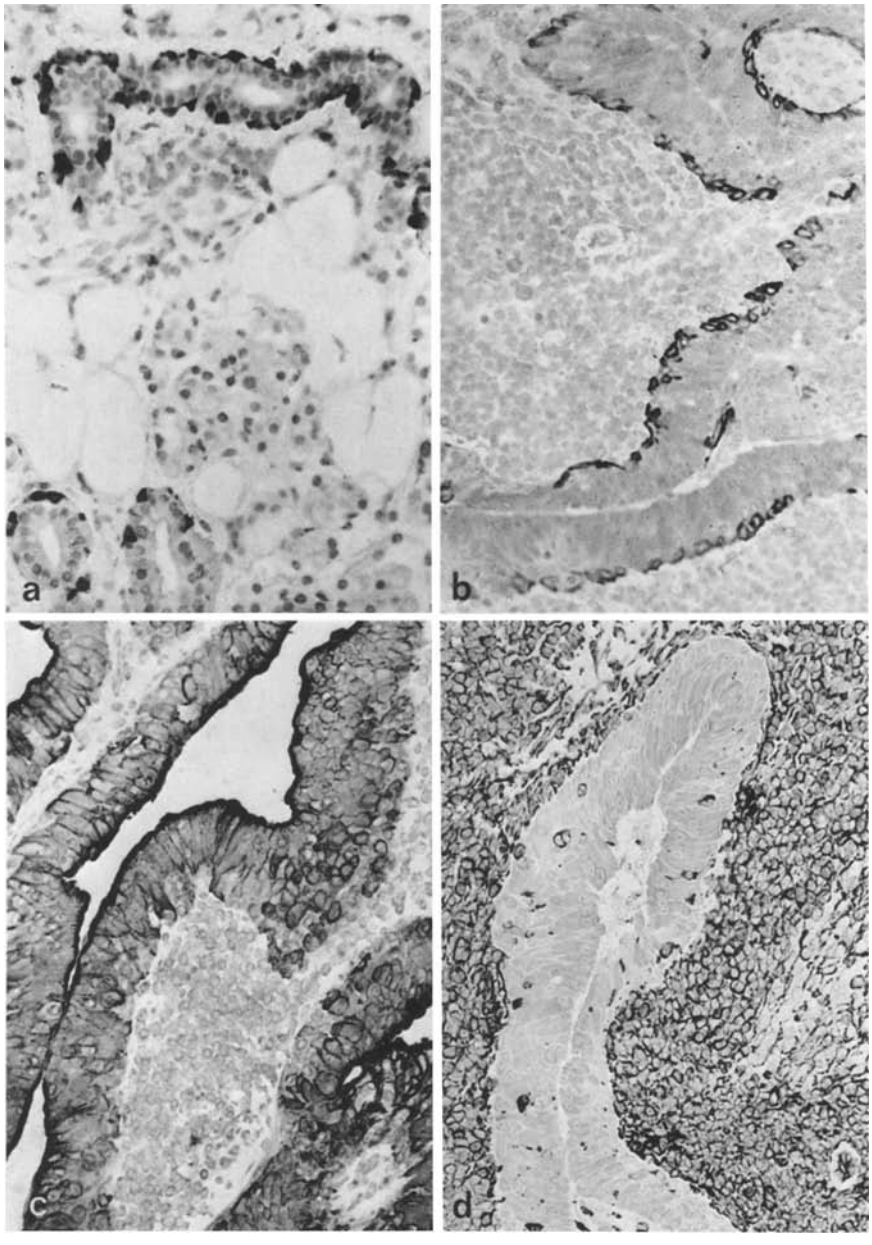

Fig. 3. a, b *KS 8.58* exhibits a selective staining of basal cells both in normal human parotid gland (**a**) and in cystadenolymphomas (**b**); **c** the epithelial compartment of cystadenolymphoma express CK 19 (AE 53-B/A2); **d** in contrast, vimentin immunoreactivity is found within the lymphoid stroma and interepithelial lymphocytes. Biotin-streptavidin, AEC; haematoxylin counterstain. Original magnification: 25×

react with and therefore specifically characterize individual epithelial cell types. Cytokeratins Nos. 7 and 18, as defined by two monoclonal antibodies, are found in serous and mucous acinar cells as well as duct epithelium. Cytokeratins 8 and 19 exhibit a strong reaction within the duct epithelium; these are, however, only weakly positive or more often absent in acinar epithelium. Without a biochemical analysis after microdissection of acinar epithelium we cannot exclude the possibility that the weak reaction with the monoclonal antibody LE 41 against cytokeratin polypeptide No. 8 is due to the antibody used. The specific expression of CK 19 within the duct epithelium confirms recent data (Geiger et al. 1987). In myoepithelial cells of the acinus, especially cytokeratins No. 19 can be unequivocally demonstrated. Our approach on normal human salivary glands completes and extends the work of Geiger et al. (1987) in that we were able to immunocytochemically demonstrate the distribution of simple epithelium cytokeratin polypeptides Nos. 7, 8, 18 and 19 by the use of monoclonal antibodies.

In human parotid, but not in submandibular glands, the co-expression of GFP and vimentin is characteristic for this cell type (see also Achtstätter et al. 1986). Nakazato et al. (1982; 1985) were the first to describe GFP immunoreactivity in pleo-

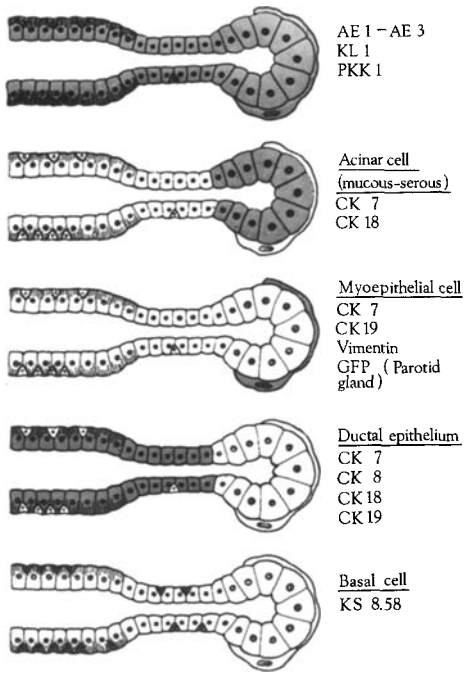

Fig. 4. Intermediate filament proteins in the human salivary gland. Selective characterization of individual cell types of the secretory unit

morphic adenomas, but failed to detect the glial filament in normal human salivary glands. The finding of GFP in non-glial cell types is extremely rare and is not yet understood (for reviews, see: Achtstätter et al. 1986; Budka 1986; Schwechheimer 1986). The additional occurrence of cytokeratin polypeptides in myoepithelial cells, originally quoted by Franke et al. (1980), indicates the extremely rare event of a triple expression of three different IF types. To date, the occurrence of three different IF types has also been described in pleomorphic adenomas of the salivary gland (Achtstätter et al. 1986), in plexus papillomas of the brain (Schwechheimer 1986) and in one unique case of papillary meningioma (Budka 1986). The meaning of such unusual IF expression in one cell type can be explained only if the regulatory mechanisms and functions of IF polypeptides are well understood.

In accordance with previous data (Geiger et al. 1987), the basal cells in both the normal salivary gland and cystadenolymphomas were selectively labeled by the monoclonal antibody KS 8.58.

In cystadenolymphomas, both the broad-spectrum cytokeratin antibodies and the panel of selective cytokeratin antibodies against simple epithelium cytokeratins Nos. 7, 8, 18 and 19 react with the epithelial compartment of the tumour. As in the normal salivary gland, basal cells of cystadenolymphomas are strongly positive with antibody KS 8.58. Our previous investigations have shown that peanut agglutinin is also a specific marker for basal cells in both the normal human parotid and submandibular gland and in cystaneolymphomas (Born et al. 1986). As basal cells represent a special cell type of the duct system and are absent from the acinar compartment, the specific demonstration of this cell type by two independent marker proteins confirm the theory that the epithelial part of cystadenolymphomas is derived from the duct system.

Acknowledgements. Monoclonal antibody KS 8.58 was Kindly provided by Dr. B. Geigu, Rehovot, Israel. We are grateful to B. Matzke and M. Kaiser for expert technical assistance, J. Moyers for photographic work and to I. Alfermann for carefully typing the manuscript.

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Accepted July 9, 1987