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A monoclonal antibody that detects myoepithelial cells in exocrine glands, basal cells in other epithelia and basal and suprabasal cells in certain hyperplastic tissues*

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Summary. Myoepithelial and luminal cells of human exocrine glands can be positively identified with two different monoclonal antibodies. Myoepithelial cells including those of the salivary gland, mammary gland and sweat gland are positively identified by an antibody CKB1. This antibody does not stain luminal cells, but stains the basal cell layer of certain human stratified epithelia and a few basal cells in simple epithelia. Thus myoepithelial cells and basal cells have certain common features. Luminal cells can be positively stained with the CK5 monoclonal keratin antibody specific for keratin polypeptide 18; this antibody does not stain myoepithelial cells. Of interest is that CKB1 also appears to stain basal and suprabasal cells in certain hyperplastic conditions.

Key words: Myoepithelial cells – Monoclonal antibodies – Keratin – Salivary glands – Mammary glands

Introduction

Myoepithelial cells are a special class of cells typically found in some exocrine glands (Mylius 1960; Hamperl 1970). These cells are thought to have contractile potential (Linzell 1955; Pinkstaff 1980) to be involved in basal membrane production (Hamperl 1970) and possibly to function as basal or reserve cells (Hume 1983; Steel and Stephens 1983).

Dedicated to Prof. Dr. F.H. Caselitz on the occasion of his 65th birthday

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Since the identification of myoepithelial cells is sometimes difficult by conventional light microscopy (Pinkstaff 1980), several methods have been introduced to try to identify these cells specifically. Histochemical techniques (Garrett and Harrison 1971; Puchtler et al. 1974) have been disappointing since neither alkaline phosphatase nor ATPase are unique markers for *all* myoepithelial cells in all glands and species (Pinkstaff 1980). Electron microscopic assays (Mylius 1960; Hübner et al. 1971; Seifert and Donath 1976) while demonstrating the characteristic ultrastructural features of myoepithelial cells are time consuming and do not always allow an unambiguous identification of this cell type. Immunohistochemical techniques (Bussolati et al. 1980) have demonstrated in particular that myoepithelial cells are strongly stained by certain actin antibodies, but again this feature does not allow unique identification of myoepithelial cells.

In the last five years, antibodies against intermediate filaments have yielded information of value in deciding on the histogenetic origin of cell types in complex tissues and neoplasms (Franke et al. 1982; Holtzer et al. 1982; Osborn et al. 1982, 1984). Myoepithelial cells contain the keratin type of intermediate filaments consistent with their epithelial nature (Franke et al. 1980). Two-dimensional analysis of keratins from different tissues has shown that in the human around 20 different keratins can be distinguished by their molecular weights and isoelectric points and have allowed the construction of catalogues in which different epithelia can be characterized by different keratin subsets (Moll et al. 1982; Wu et al. 1982). The exact keratin complement of myoepithelial cells has, however, not been determined. Parallel studies using monoclonal antibodies which recognize only one or a few keratin polypeptides have also yielded information on the keratin content of particular epithelial cell types (Debus et al. 1982, 1984; Tseng et al. 1982; Lane 1982). Their use offers new possibilities in subtyping epithelial tissues (reviewed in Cooper et al. 1984).

Here we describe a monoclonal antibody which has a special affinity for myoepithelial cells in exocrine glands. Interestingly, it also stains the basal cell layer in several stratified epithelia as well as some basal cells in certain simple epithelia.

Materials and methods

The human tissues listed in Table 1 were obtained during surgery and were immediately quick frozen in liquid nitrogen. 5 μ sections were cut on a Reichert cryostat OM U. Some were stained directly and others after lyophilization at −20° C. In both instances specimens were fixed by immersion in acetone for 10 min at −10° C.

The following mouse monoclonal antibodies were used: 1. Lu5 is a broad specificity keratin antibody as judged by immunofluorescence results (von Overbeck et al. 1985; our unpublished results). Its specificity by immunoblotting has not been reported. It was obtained from Dr. C. Stähli, Central Research Division, F. Hoffmann-La Roche and Co. Ltd., Basel, Switzerland.

2. CK5 is similar to the CK1–CK4 keratin monoclonals (Debus et al. 1982) in that it specifically recognizes the keratin 18 polypeptide characteristic of simple epithelia in immunoblots of two-dimensional gels (Tölle et al. 1985).

Table 1. Immunohistochemical results with monoclonal antibodies CK 5 and CK B1

Tissue	Number of cases	Type of cells					
		Acinic cells	Ductal cells	Myoepithelial cells	Basal cells	Intermediate cells	Upper cells
Distribution of positive cells using antibody directed against *keratin 18 (CK5)*							
Parotid gland	5	+	+	−			
Submandibular gland	5	+	+	−			
Mammary gland	5	+	+	−			
Sweat gland	3	+	+	−			
Pancreas	3	+	+				
Prostate gland	3	+	+	−			
Cervical epithelium	3				+[a]		
Laryngeal epithelium	3				+[a]		
Distribution of positive cells using antibody *CK B1*							
Parotid gland	5	−	−	+			
Submandibular gland	5	−	−	+			
Mammary gland	5	−	−	+			
Sweat gland	3	−	−	+			
Pancreas	3	−	−				
Prostate gland	3	−	−	+			
Cervical epithelium	3				+	−	−
Laryngeal epithelium	3				+	−	−

[a] Cuboid cells in the glandular epithelium are positive for keratin 18

3. CKB1 is a monoclonal antibody isolated from a fusion of spleen cells of Balb/c mice immunized with human callous keratins with the PA1 myeloma line (Osborn et al. in preparation).

Monoclonal antibody reaction was detected either in the indirect immunofluorescence technique with FITC-labeled goat anti-mouse IgGs (Capell Laboratories, Cochranville, PA, USA or using biotin-labeled goat anti-mouse IgGs and the Vectastain Kit (Vector Laboratories, Burlinghame CA, USA). Peroxidase was visualized by the diaminobenzidine reaction.

Results

The results obtained by staining various tissues are summarized in Table 1. The staining with all three monoclonal antibodies was restricted to epithelial cell types. Stromal cells, and other non epithelial cells were not stained by Lu5, by CK5 or by CKB1.

Normal parotid and submandibular gland

The normal parotid and submandibular gland contains different epithelial cell types: acinic cells, duct cells (intercalated duct cells, striated duct cells)

and myoepithelial cells. Some authors also distinguish a fourth epithelial cell type, the so-called "indifferent cells" (for references see Introduction).

Lu5. Lu5 decorated all epithelial cells (Fig. 1a).

CK5. The antibody CK5 indicates the presence of keratin 18. The acinic cells and the duct cells were positive whereas myoepithelial cells were not stained.

CKB1. The cells which were positive with this antibody were different from those stained by the cytokeratin 18 specific antibody CK5. CKB1 did not stain either acinic cells or the normal duct cells. It did stain the basal cells which line the epithelial row of ductal cells. The myoepithelial cells, the basket-like cells at the periphery of the acini and the ducts, were positive. In the region of the striated ducts, the basal cells display a more triangular shape and the branches are not so prominent as those of the basket cells but they were clearly stained by CKB1 (Fig. 1b).

Mammary gland

In the mammary gland, the terminal duct system as well as the larger duct system can be distinguished. Both have myoepithelial cells. Thus there is some analogy with the salivary glands, although functional differences exist between the two tissues – for instance hormonal influences on the mammary gland.

Lu5. Lu5 decorated all epithelial cells. Thus the small and the larger ductal systems were decorated, as were the myoepithelial cells.

CK5. CK5 decorated a subset of epithelial cells, i.e. the "acinic" cells and the duct cells, whereas the myoepithelial cells were negative or were only very weakly stained (Fig. 1c).

CKB1. This antibody did not react with the epithelial cells lining the ductal lumen. The true epithelial cells of the smaller and the larger ducts were generally negative for this antibody. In contrast, the myoepithelial cells were positive. These cells sometimes display a basket-like appearance around the terminal ducts. The larger ducts showed basal cells of triangular shape, which were positively stained by this antibody (Fig. 1d).

Sweat glands

The architecture of the sweat glands follows that of the other exocrine glands in that ductal cells and myoepithelial cells can be distinguished.

Lu5. All epithelial cells of the sweat glands were positive.

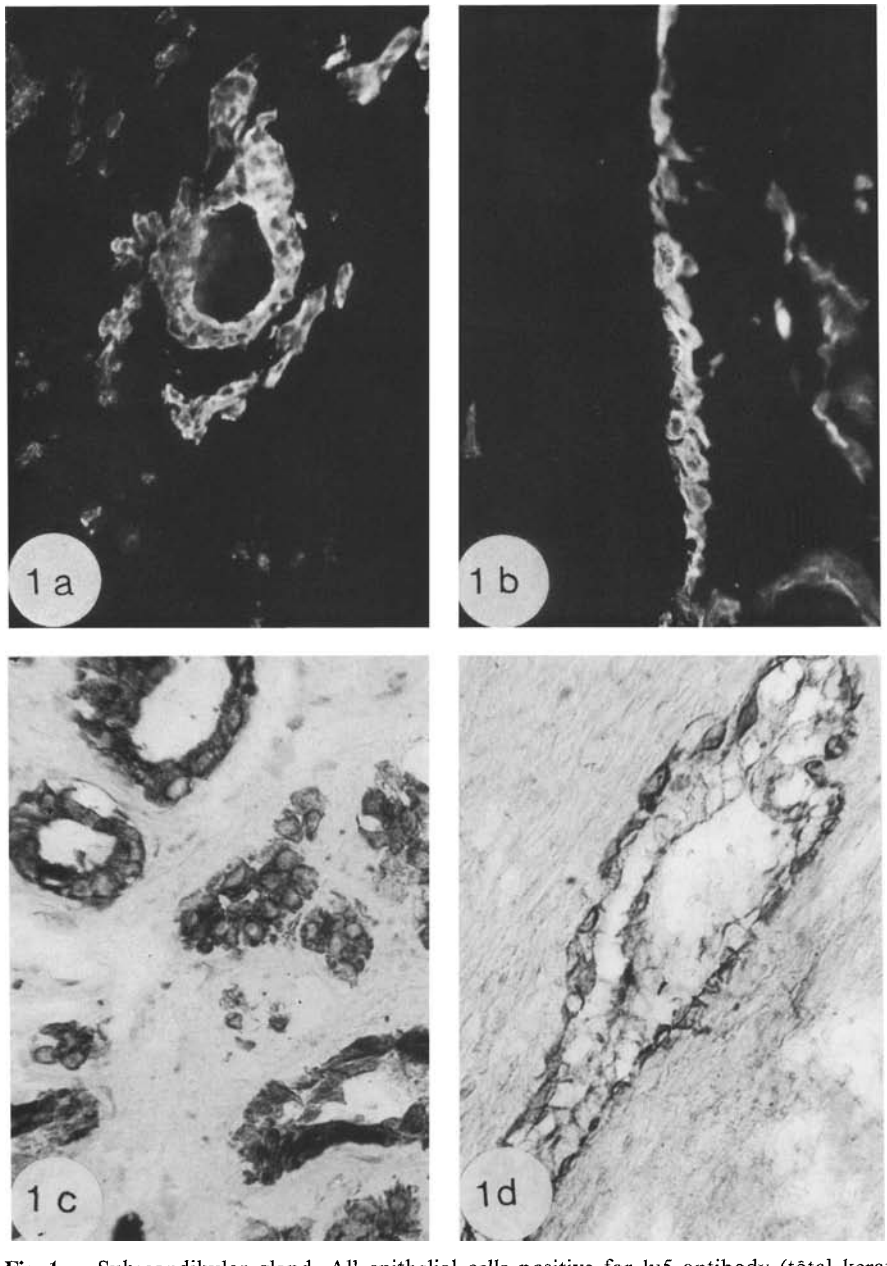

Fig. 1. a Submandibular gland. All epithelial cells positive for lu5 antibody (tōtal keratin). Stromal part negative. Indirect immunofluorescence with antibody lu5. **b** Submandibular gland. Positive cells at the peripheral layer of the larger ducts. Luminal epithelial cells negative. Indirect immunofluorescence with antibody CKB1. **c** Mammary gland. Ductal cells positive with an antibody against cytokeratin 18 (CK5). Myoepithelial cells negative. No staining of the stroma. Immunoperoxidase (Avidin-Biotin-System). **d** Mammary gland. Positive myoepithelial cells lining a larger duct. Epithelial ductal cells in the lumen are negative for CKB1. Immunoperoxidase (Avidin-Biotin-System). × 300

CK5. This antibody decorated the ductal cells of the sweat glands. Only the epithelial cells at the border of the lumina were positive for this antibody. The myoepithelial cells were generally negative.

CKB1. This antibody did not label the "normal" ductal cells. There was a strong staining of myoepithelial cells in the sweat glands. These cells surrounded the ductal elements in the typical basket-like manner.

Pancreatic gland

The architecture of the exocrine part of the pancreatic gland is similar to that of the parotid gland. There are acinic cells and duct cells, but in contrast to the parotid gland, no myoepithelial cells are found in the pancreas. Therefore this organ was of particular interest.

Lu5. All epithelial cells were positive for this antibody. The acinic cells and the ductal cells were clearly stained.

CK5. All epithelial cells of the pancreas were positive for this antibody. Acinic and ductal cells were stained equally (Fig. 2a).

CKB1. No staining of epithelial cells was obtained (Fig. 2b).

Prostate gland

The prostate is composed of tubulo-aveolar glands and a more or less developed fibromuscular stroma. The glands are surrounded by a cuboid or columnar epithelium. At the basal part of the gland, some triangular shaped cells can be identified.

Lu5. There was a strong staining of the epithelial part. Both normal and cystic dilated glands were positive (Fig. 2c).

CK5. The staining with this antibody was similar to that of lu5. The epithelial part was clearly stained (Fig. 2d).

CKB1. Peripherally located triangular shaped cells were positively stained. The staining seemed to be especially intense in the cystic dilated parts of the glands (Fig. 2e).

Cervix uteri

The cervix uteri is a zone where there is transition from squamous to cuboidal epithelium. In this part, metaplasia is often observed.

Lu5. Both the squamous epithelium and the cuboid epithelium were positive (Fig. 3a).

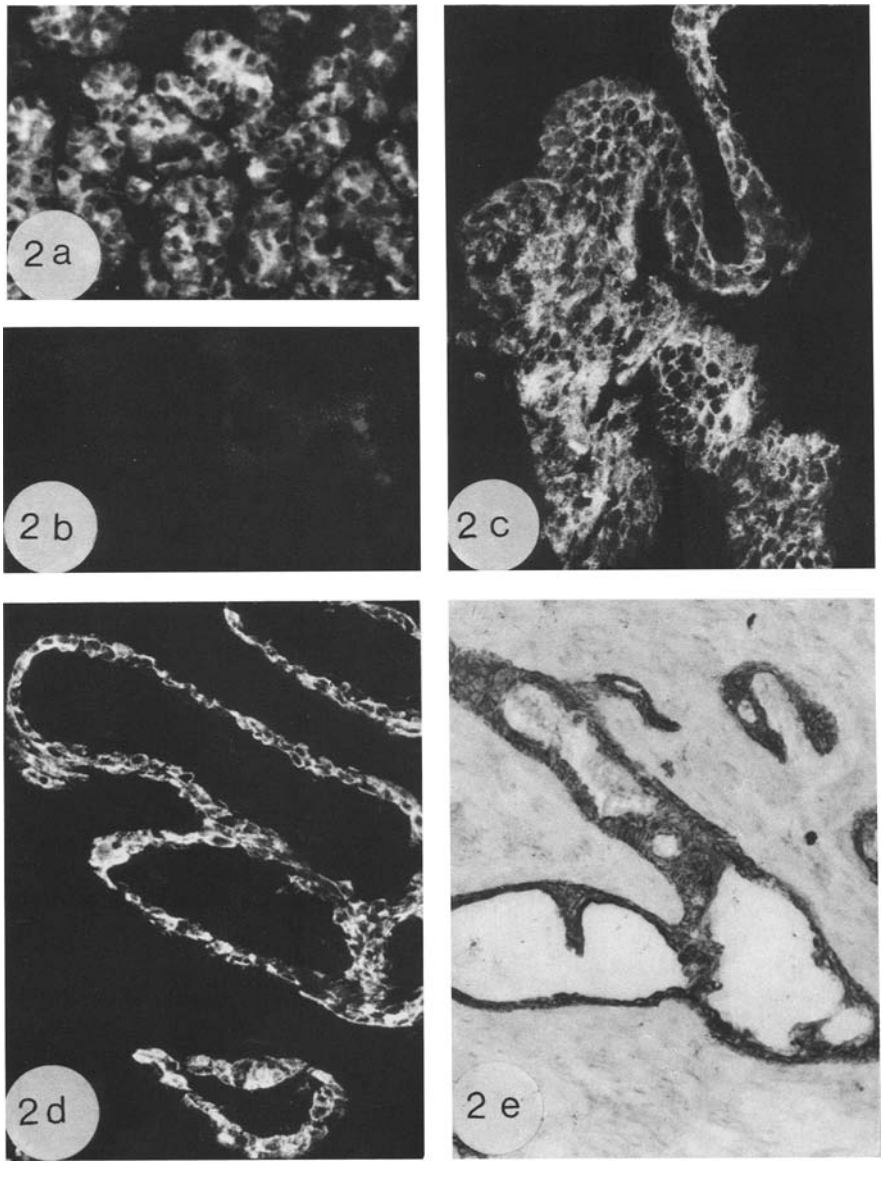

Fig. 2. a Pancreas. Cytokeratin 18-positive acinic and ductal cells staining with CK5. Stromal part negative for this antibody. Indirect immunofluorescence. **b** Pancreas. No staining of the acinic and ductal cells with CKB1. Indirect immunofluorescence with antibody CKB1. **c** Prostate gland. All epithelial cells positive with a broad specificity keratin antibody (lu5). Stromal part negative. Indirect immunofluorescence with antibody lu5. **d** Prostate gland. Ductal cells at the lumina positive for cytokeratin 18 (CK5). Immunoperoxidase (Avidin-Biotin-System). **e** Prostate gland. Peripheral layer of cells positive with CKB1. Stromal part clearly negative. Indirect immunofluorescence. ×120. **a, b** ×300, **c–e** ×120

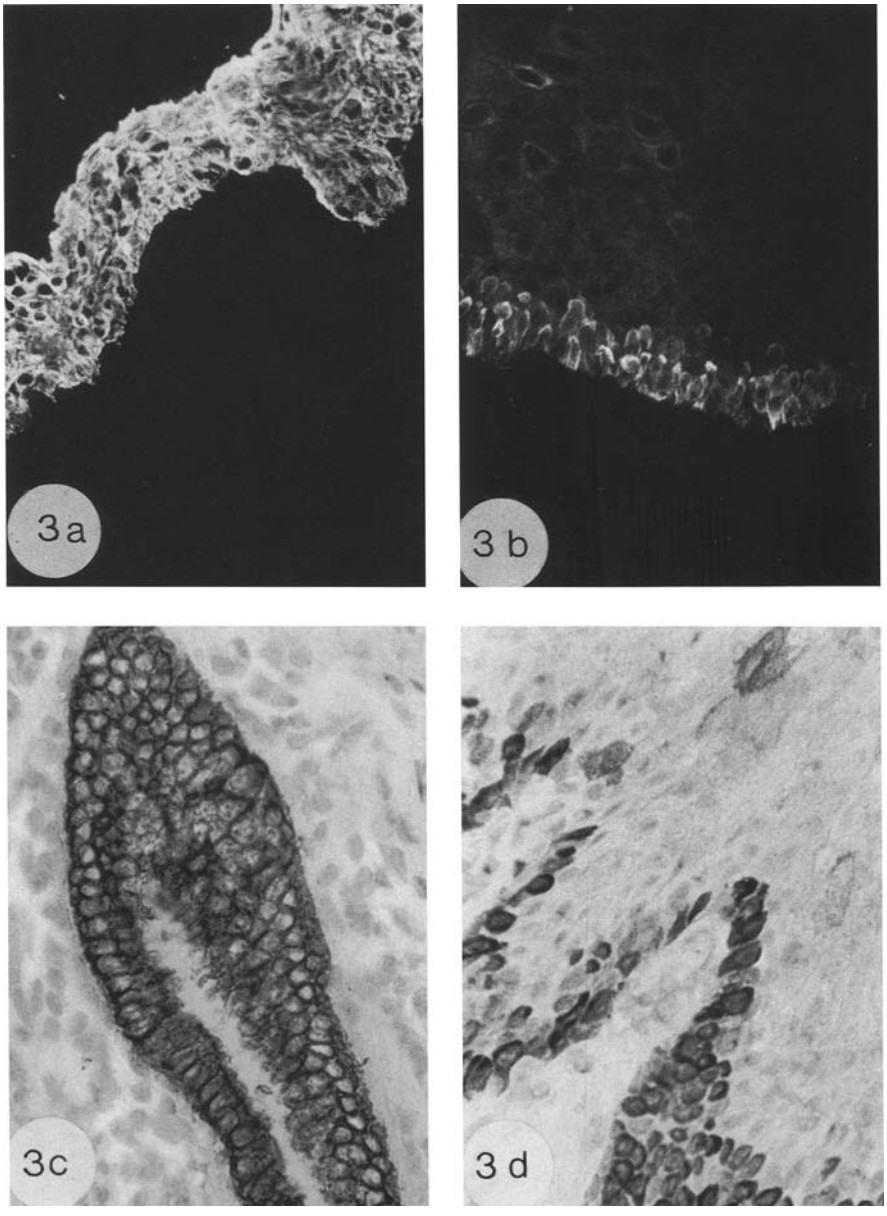

Fig. 3. a Cervix uteri. All cells of stratified epithelium positive for Lu5. Indirect immunofluorescence with lu5. **b** Cervix uteri. Only weak staining of cells in the stratified epithelium. Indirect immunofluorescence with CK5. **c** Cervical gland. Strong staining of cuboidal epithelium with antibody against cytokeratin 18 (CK5). Immunoperoxidase (Avidin-Biotin-System). **d** Cervix uteri. Part of squamous epithelium. Strong staining of the basal cell layer with CKB1. No staining of the cells in the upper layers. Immunoperoxidase (Avidin-Biotin-System). **a, b** $\times 120$, **c, d** $\times 300$

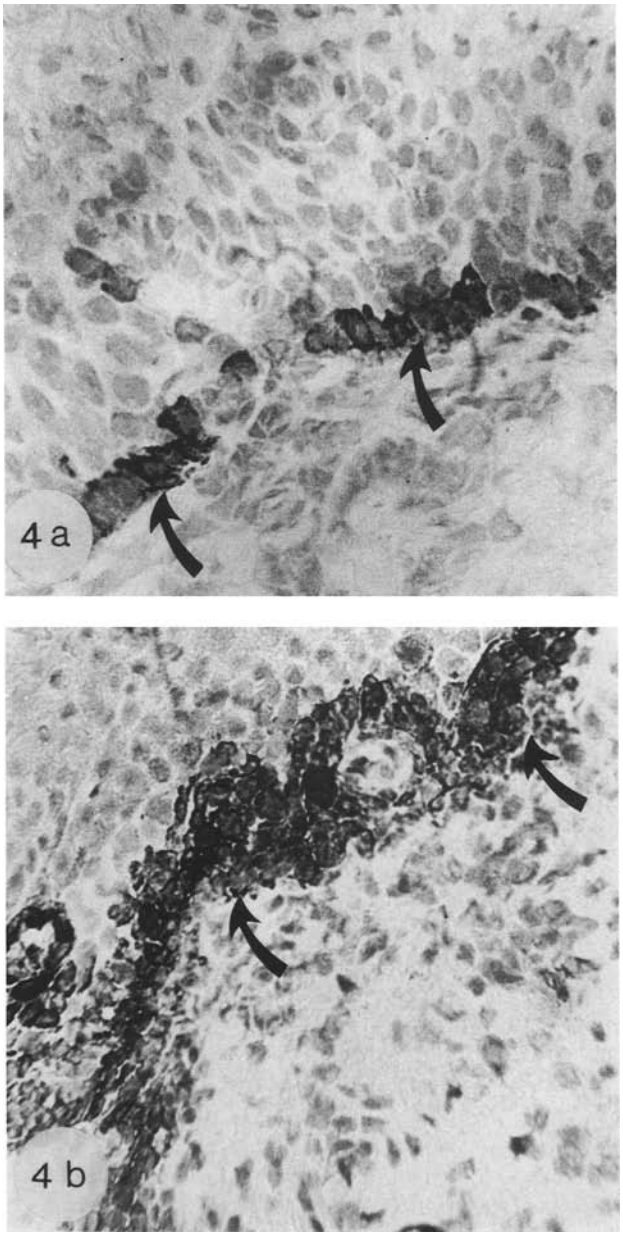

Fig. 4. a Larynx. Stratified epithelium. Strong staining of the basal cell layer with antibody CKB1 (*arrows*). Negative cells in the upper layer. Immunoperoxidase (Avidin-Biotin-System). **b** Larynx. Stratified epithelium. Strong staining of the basal cells, (arrows) and the adjacent layer in a dysplastic lesion of the squamous epithelium with antibody CKB1. Immunoperoxidase (Avidin-Biotin-System). ×120

CK5. Generally, clear staining was seen in the cuboidal cervical cells (Fig. 3c). The adjacent squamous epithelium was only partly positive and in general more weakly stained (Fig. 3b).

CKB1. Basal cells in the glandular epithelium and in the squamous epithelium were positive. They were clearly detected as a row lining the basal membrane (Fig. 3d). In particular, strong staining was observed at the border of the glandular cervical epithelium.

Larynx

In the region of the larynx, there is a transition between two kinds of epithelium, the squamous epithelium and the simple glandular epithelium. In some senses, the situation is similar to that seen in the cervix uteri.

Lu5. Both kinds of epithelium were positively stained. The staining of the squamous epithelium seemed to be somewhat more intense than that of the cylindric epithelium.

CK5. Only the cylindrical epithelium was stained in a strong manner. On the other hand, there was only a faint staining of some cells of the squamous epithelium.

CKB1. The basal cells were positive (Fig. 4a). They displayed the typical localization and the typical shape. In some cases, there seemed to be a hyperplasia of basal cells and in these instances multilayered staining was seen (Fig. 4b).

Discussion

The presence of keratins in myoepithelial cells has been demonstrated in animal exocrine glands by Franke et al. (1980) and in human salivary glands by Caselitz et al. (1981). These studies used broad specificity polyclonal antibodies raised against keratin, or a prekeratin fraction, so that a wide variety of different epithelial cells were detected. Nathrath et al. (1982) reported a polyclonal antibody raised against a human callous fraction rich in keratins which displayed restricted reactivity on human adult epithelia and which detected myoepithelial cells but not luminal cells. A conventional antibody with apparently similar specificity for myoepithelial cells, except that it reacted only with mouse tissues has been reported by Asch et al. (1981).

With the advent of monoclonal antibodies in the field of intermediate filaments it has become possible to further subdivide epithelia according to their keratin content (Tseng et al. 1982; Moll et al. 1982; Wu et al. 1983) and to determine whether the results obtained with appropriately characterized monoclonal antibodies agree with those obtained by two dimensional gel electrophoresis of tissue homogenates (Debus et al. 1984). Thus for ex-

ample it was possible to show agreement when different human tissues and tumours were studied by immunofluorescence microscopy with a monoclonal antibody specific for keratin 18, and with the presence of the polypeptides as assayed by two dimensional gel electrophoresis (Bartek et al. 1985; Debus et al. 1982, 1984). Recent studies with monoclonal antibodies specific for instance for keratin 7, or keratin 8, or keratin 19 have extended this approach (Tölle et al. 1985; Bartek et al. 1985; Nagle et al. 1985). Once such antibodies have been appropriately characterized they allow the identification and characterization of the keratin content of even single cells present in complex tissues or in carcinomas. In particular antibodies specific for keratins 18 and 19 have been shown to bind to luminal cells but not to myoepithelial cells of the breast (Debus et al. 1982, 1984; Bartek et al. 1985; Nagle et al. 1985).

In the current study we have described the isolation and characterization of a monoclonal antibody CKB1 which specifically stains myoepithelial cells in human exocrine glands, but does not stain the luminal cells. As shown by results on a variety of human tissues, myoepithelial cells can be positively identified in salivary glands (parotid and submandibular), in mammary glands and in sweat glands. Interestingly two kinds of cells were detected by antibody CKB1. Those cells surrounding the terminal ductal system (especially the acinic cells) had long branches of cytoplasm which justifies their designation as basket cells (Hamperl 1970; Pinkstaff 1980). The cells surrounding the larger ducts had a more compact cytoplasm and a triangular shape. They abut on to the basal part of ductal cells. These cells were also positive with the CKB1 antibody.

In this investigation we not only tested those organs where myoepithelial cells are expected, but also studied other glands such as the prostate gland and the pancreatic gland. The prostate gland was positive for CKB1. The positive cells were basally located, and were triangular in shape. In the course of hyperplasia of the prostate gland CKB1 seemed to detect an augmentation of these basally located cells in some parts of the tissue (Fig. 2). The cells of the pancreatic gland although positive for keratin and especially for keratin 18 were negative when tested with the antibody CKB1. In this context the exocrine part of the pancreas may be regarded as a negative control for CKB1. The pancreas is characterized by the absence of myoepithelial cells and in agreement with this the antibody CKB1 did not react with the parenchymal tissue.

For comparative reasons we also investigated tissues where there is a transition between squamous epithelium and glandular epithelium. Often in such parts areas of metaplasia arise, a fact which points to an involvement of the basal cell layer (Koss 1979). Both the cervical and laryngeal epithelium although different in some respects gave similar results. The epithelium was positive with lu5. The glandular parts composed of cuboid cells were positive with lu5 and with CK5. A few cells in the adjacent squamous epithelium also showed a reaction with CK5. The most interesting finding was that in both tissues the basal cells were positive for the antibody CKB1. Thus, although these cells do not fit the usual definition of myoepithelial

cells, they seem to share some common characteristics with myoepithelial cells. Generally in normal tissues there was only one layer of cells positive for CKB1. Perhaps this result points to a similar functional role of the basal cell system both in squamous and in glandular epithelium. The common function could perhaps be seen in the role as stem cells (Hume 1983, Steel and Stephens 1983).

Further investigations have to identify the polypeptide recognized by the CKB1 antibody. A comparison of the staining patterns with the known keratin content of different epithelial cell types (Moll et al. 1982) might suggest either keratins 5 or 17 as possible candidates. However CKB1 does not stain Hela cells suggesting that it is not directed against keratin 17. Immunoblotting experiments have thus far not given positive result on tissues such as hair follicles or epidermal keratins which should be rich in keratin 5. Thus it is still an open question whether CKB1 recognizes a keratin polypeptide or another polypeptide shared by the cell types described.

The antibody CKB1 may have value in certain hyperplastic and neoplastic conditions. Thus, for instance in the prostate gland, cells were especially intensely stained in those cystic dilated areas found during the course of benign prostate hyperplasia (e.g. Fig. 2e). In some reactive conditions – e.g. in inflammative and dysplastic lesions of cervix and larynx – not only the basal cells, but also additional suprabasal cell layers were positive although less strongly stained by CKB1, thus perhaps providing an immunological assay for these conditions. In the case of scleradenosis, a proliferative disorder of myoepithelial cells that may be difficult separate from tubular carcinoma, the positive reaction with CKB1 would be a clear argument in favor of scleradenosis, and thus for the benign nature of the lesion in question. In some cases of mammary cancers it may be difficult to distinguish between preinvasive and invasive tumors (Murad and van Haam 1968, Ahmed 1974). In these instances it would be helpful to outline the row of myoepithelial cells. An intact row of these cells would indicate the still preinvasive nature of this tumor.

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