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Differentiation

Monoclonal antibodies to various acidic (type I) cytokeratins of stratified epithelia

Selective markers for stratification and squamous cell carcinomas

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Abstract. We determined the reactivity of two monoclonal antibodies to cytokeratins that are typically expressed in certain stratified epithelia and several human squamous cell carcinomas using immunoblotting techniques and immunofluorescence microscopy. Antibody K_S 8.12 reacted specifically with cytokeratin polypeptides nos. 13 and 16, and stained noncornified squamous epithelia in a rather uniform way. The examination of diverse human carcinomas showed all squamous cell carcinomas to be positively stained with this antibody, whereas all adenocarcinomas were negative. Another antibody, K_K 8.60, reacted with polypeptides nos. 10 and 11, and uniformly stained the suprabasal layers of the epidermis. In several noncornified squamous epithelia (e.g., tongue, exocervix), in thymus reticulum epithelial cells, and in moderately and well differentiated squamous cell carcinomas this antibody exhibited a nonuniform labeling pattern that allowed the detection of individual cytokeratin-10/11-positive cells scattered throughout the tissue. It is concluded that antibodies K_S 8.12 and K_K 8.60 represent specific molecular probes for the definition of certain stages of squamous differentiation in normal development as well as in pathological processes such as squamous metaplasia and carcinogenesis. We propose the use of these antibodies in the differential diagnosis of carcinomas and their metastases.

Introduction

Extensive studies performed in recent years have established the usefulness of intermediate-sized (7–11 nm) filaments (IFs) as cell-type markers in histology and tumor diagnosis [1–6, 9, 13–16, 26–29, 36–42, 47, 51]. Such studies have developed in two major phases. Antibodies specific to the five major classes of IF proteins (cytokeratins, vimentin, desmin, glial filaments, and neurofilaments) have been successfully applied as specific immunohistochemical group markers that distinguish between cells derived from epithelial, mesenchymal, myogenic, glial, or neural origin. Following the finding that, in contrast to the other IF families which consist of one or only a few polypeptides, the cytokeratins comprise a repertoire of approximately 19 distinct polypeptides expressed in different combinations in the diverse human epithelia [8, 11, 15, 20–24, 29, 31, 32, 39, 45, 47, 49–52], a second phase of IF research has aimed at the differential identification of various epithelia, as well as of tumors derived therefrom, using the different cytokeratin polypeptides as cell-type-specific criteria. For the determination of the different polypeptides expressed in a given epithelial tissue, whether it be normal or transformed, two complementary experimental approaches have been used, i.e., biochemistry and immunohistochemistry. Biochemical studies have generally been based on the electrophoretic separation of individual cytokeratin polypeptides present in different kinds of epithelial cells [6, 11, 15, 29–32, 45, 47, 49]. Alternatively, specific cytokeratins can be identified in situ by immunohistochemical staining using antibodies that exhibit a defined specificity for certain cytokeratin polypeptides [8, 10, 19, 26, 36–38, 49, 50–52]. Conceivably, using a battery of different antibodies (preferably monoclonal) of the desired specificities, the latter approach may make it possible to identify groups of cytokeratins or even individual polypeptides with a high level of both polypeptide specificity and microscopic resolution.

In the present study, we used different monoclonal antibodies to identify and localize certain cytokeratins present in squamous epithelia as well as in various carcinomas exhibiting a squamous morphology. The results of our study indicate the widespread occurrence of the antigenically related cytokeratin polypeptides nos. 13 and/or 16 in nonepidermal stratified epithelia and in squamous carcinomas. In contrast, cytokeratins nos. 10 and/or 11 (detected by another antibody, K_K 8.60), which are detected in upper layers of the epidermis, were also found in cornified regions or individual cells of other epithelia and in certain squamous cell carcinomas.

Methods

Tissues and cells. All tissues examined were obtained during surgery and processed immediately. The tissues were either frozen in liquid-nitrogen-cooled isopentane [13, 14] or were fixed in ethanol and embedded in paraffin [1]. The histopathological diagnosis of all tumors examined was carried out independently using hematoxylin-and-eosin (HE)-stained sections of parallel formalin-fixed, paraffin-embedded tissues.

The cultured cells used included human epidermoid carcinoma line A-431 (maintained in culture in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum; cf. [29]) and human bladder carcinoma line RT-4 [43].

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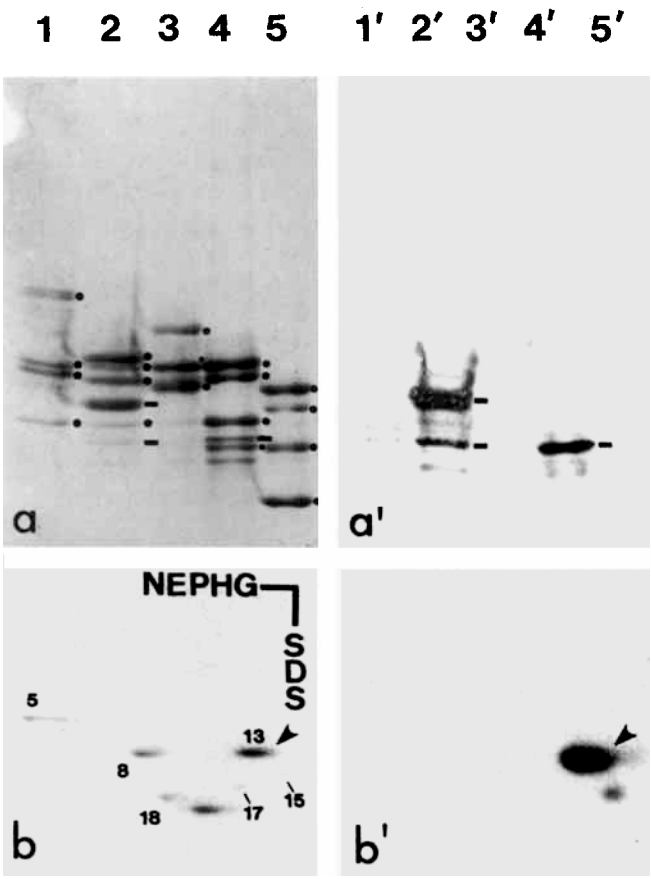


Fig. 1 a, a', b, b'. Characterization of monoclonal antibody K_S 8.12 by immunoblotting. **a** Coomassie-blue-stained polypeptides revealed by SDS-polyacrylamide gel electrophoresis of cytoskeletal proteins from various human cells and tissues. The major cytokeratin polypeptides are designated by *dots*, and the positively reacting polypeptides are identified by *bars*, listed from the *top* to the *bottom*. *Slot 1*, epidermis (cytokeratin nos. 1, 5, 10/11, 14/15); *slot 2*, tongue epithelium (nos. 4, 5, 6, 13, 14, 16); *slot 3*, corneal epithelium (nos. 3, 5, 12); *slot 4*, hair follicles, primarily root-sheath material (nos. 5, 6, 14, 16, 17; actin); *slot 5*, cultured bladder carcinoma cells of line RT-4 (nos. 7, 8, 17/18, 19). **a'** Autoradiograph showing an immunoblot of a parallel gel. The positive bands are visualized by horseradish-peroxidase-coupled rabbit antimouse immunoglobulins. Note the positive reaction of cytokeratins nos. 13 and 16 in *slots 2'* and *4'* (these cytokeratin polypeptides are designated by *bars* in **a**). **b** Coomassie-blue staining of polypeptides seen after two-dimensional gel electrophoresis of cytoskeletal proteins of A-431 cells. *NEPHG*, first-dimension nonequilibrium pH-gradient electrophoresis; *SDS*, second-dimension electrophoresis in the presence of SDS. **b'** Corresponding immunoblot of a parallel gel. The antibody-binding polypeptides are visualized by ^{125}I-coupled goat antibody directed against mouse immunoglobulins followed by autoradiography. Note the strong decoration of cytokeratin no. 13 (*arrowheads* in **b** and **b'**); a minor spot in the area of cytokeratin no. 15 is also detectable (for discussion, see text)

Fig. 3. Schematic representation of the polypeptide specificities of the three monoclonal antibodies used. ●, polypeptides strongly reactive with antibody K_G 8.13 (cf [19]); ◑, polypeptides showing a weak reaction with K_G 8.13; ▲, polypeptides reacting with K_S 8.12; □, polypeptides reacting with antibody K_K 8.60 (occasionally observed weak reactivity with polypeptide no. 1 is indicated by a *square*); ○, polypeptides that do not react with any of these antibodies. *PGK*, position of 3-phosphoglycerokinase; *BSA*, bovine serum albumin; *A*, α-actin (used for reference in co-electrophoresis).

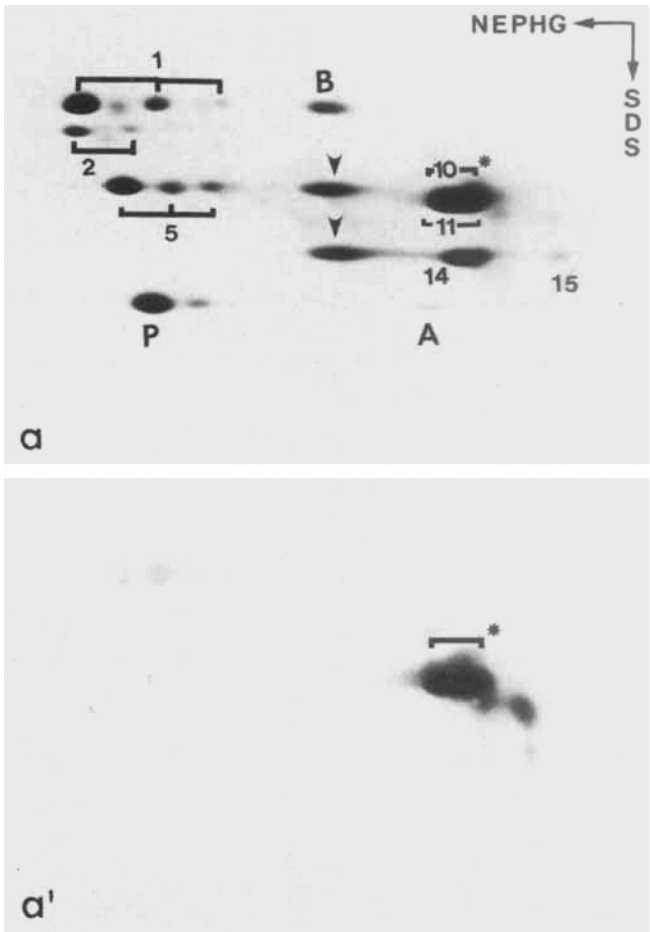
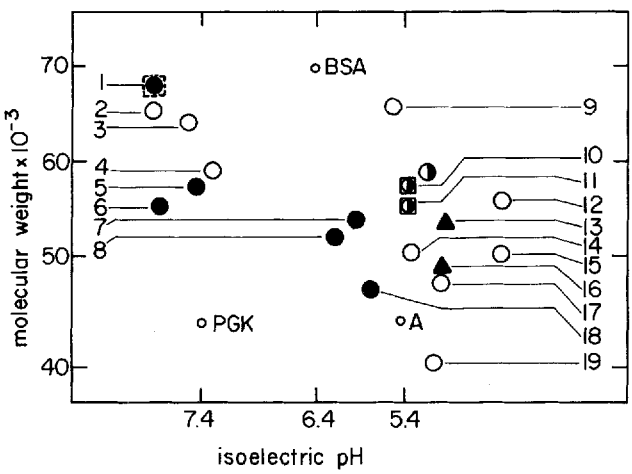


Fig. 2 a, a'. Characterization of monoclonal antibody K_K 8.60 by immunoblotting. **a** Coomassie-blue-stained polypeptides revealed after two-dimensional gel electrophoresis (for symbols, see legend to Fig. 1 b) of cytoskeletal proteins of human thigh epidermis. The marker polypeptides included for co-electrophoresis were as follows: *P*, 3-phosphoglycerokinase; *B*, bovine serum albumin. *A*, endogenous actin. The arrowheads denote the proportion of cytokeratins 5 and 14 which had migrated as a complex in the first dimension in this particular separation. **a'** Immunoblot of a parallel gel; the positive spots are visualized by peroxidase-coupled rabbit antimouse immunoglobulins. Note the strong staining of cytokeratins nos. 10 and 11 (*brackets* and *asterisks* in **a** and **a'**) and their degradation products



Antibodies. The three monoclonal antibodies used were all obtained from mice originally immunized with bovine muzzle prekeratin as described elsewhere [19]: (1) antibody K_G 8.13, a broadly cross-reactive antibody (for characterization, see [19]) which positively stained all of the epithelia and carcinomas examined; (2) K_S 8.12 (IgG_1); and (3) K_K 8.60 (IgG_1). The specificities of the last two antibodies are discussed in detail later. These antibodies were applied as culture supernatants of the hybridomas or as ascites fluids developed in BALB/c × DBA/2 Fl mice.

The secondary antibodies were affinity-purified goat antibodies raised against mouse F(ab)'2 and were conjugated to lissamine-rhodamine sulfonyl chloride [7, 18].

Immunohistochemistry. Sections of frozen tissue were cut using a Frigocut 2700 cryostat (Jung-Reichert, Nussloch, FRG) and processed as described previously [13]. Sections of paraffin-embedded tissues were deparaffinized in xylene and ethanol, rehydrated, and immunolabeled [1].

Gel electrophoresis and immunoblotting analysis. Analysis of the cytokeratin composition of various tissues and tumors was carried out using one- or two-dimensional gel electrophoresis as previously described [25, 34, 35] with minor modifications [29–32]. Relevant regions of thick (30 μm) frozen sections were dissected under a microscope. Noncytoskeletal proteins were extracted with high-salt buffer and detergent, and the cytoskeletal residue was electrophoretically analyzed [29–32]. To facilitate the identification of individual polypeptides, reference proteins, including α-actin, phosphoglycerokinase, and bovine serum albumin, were added to the samples. Immunoblotting transfer was carried out according to the method of Towbin et al. [48], and the nitrocellulose sheets were processed with primary antibodies prior to reaction either with iodinated or peroxidase-labeled secondary antibodies to mouse Ig or with iodinated protein A [19].

Results

Specificities of monoclonal antibodies K_S 8.12 and K_K 8.60

The polypeptide specificities of K_S 8.12 and K_K 8.60 were examined by immunoblotting analysis of cytoskeletal preparations from different cells and tissues. Figure 1a and a′ shows a Coomassie-blue-stained gel and the corresponding immunoblot with antibody K_S 8.12. On this gel, nearly all of the human cytokeratin polypeptides were present, but only polypeptides nos. 13 and 16 reacted positively and specifically with antibody K_S 8.12. This suggests that these two polypeptides share a unique epitope within the acidic (type I) subfamily of cytokeratin polypeptides (for nomenclature, see [8, 20, 21, 24, 46, 47]). Two-dimensional gel electrophoresis of a cytoskeletal preparation of cultured human epidermoid carcinoma cells of line A-431 (Fig. 1b and b′) revealed a positive reaction with cytokeratin no. 13 (cytokeratin no. 16 is not present in these cells) as well as in the region of cytokeratin no. 15. Due to the low amounts of cytokeratin no. 15 present, it could not be decided whether the minor extra spot detected in the immunoblot shown in Fig. 1b′ represented a cross-reaction with cytokeratin no. 15 or a breakdown product of polypeptide no. 13; such breakdown products, resulting in shifts to more acidic fragments, are found with vimentin and cytokeratin no. 8 (e.g., see [17, 44]).

Table 1. Immunohistochemical staining of normal human tissues with monoclonal antibodies to different cytokeratin polypeptides

Tissues	K_G 8.13	K_S 8.12	K_K 8.60
Skin			
Epidermis: basal layer	+	−	−
Epidermis: suprabasal layers	+	−	+
Outer root sheath of hair follicle	+	+[a]	−
Eccrine sweat glands: all elements	+	−	−
Apocrine sweat glands: all elements	+	−	−
Gastrointestinal tract			
Tongue: squamous epithelium	+	+	(+)
Salivary gland: acini	+	−	−
Salivary gland: ducts	+	±[b]	−
Esophagus: squamous epithelium	+	+	−
Stomach: mucosa	+	−	−
Large intestine: mucosa	+	−	−
Exocrine pancreas: all elements	+	−	−
Liver: hepatocytes	+	−	−
Liver: bile ducts	+	±	−
Urinary system			
Urinary bladder: urothelium	+	+	−
Urethra: urothelium	+	+	−
Female genital tract			
Vagina: basal layer	+	+	−
Vagina: suprabasal layers	+	+	(+)NU
Exocervix	+	+	(+)NU
Endocervix	+	−	−
Endometrium: all epithelial elements	+	−	−
Oviduct: mucosa	+	−	−
Respiratory system			
Trachea, bronchus: basal cells	+	(+)	−
columnar cells	+	−	−
glands	+	−	−
Lung: alveolar epithelium	+	−	−
Thymus	+	(+)[c]	(+)HB

HB, Hassall bodies; (+), a sizeable proportion of positive cells
[a] Basal layer
[b] Some individual ductal cells positive
[c] Predominantly in medullary regions, notably around Hassall bodies
NU, nonuniform labeling.

To examine the polypeptide specificity of K_K 8.60, we performed immunoblotting analysis of cytoskeletal proteins of human thigh epidermis. As shown in Fig. 2, only polypeptides nos. 10 and 11 reacted with this antibody. In some preparations as well as at very high protein loadings, a weak reactivity with cytokeratin no. 1 was also observed; this reactivity may either be nonspecific or may reflect some

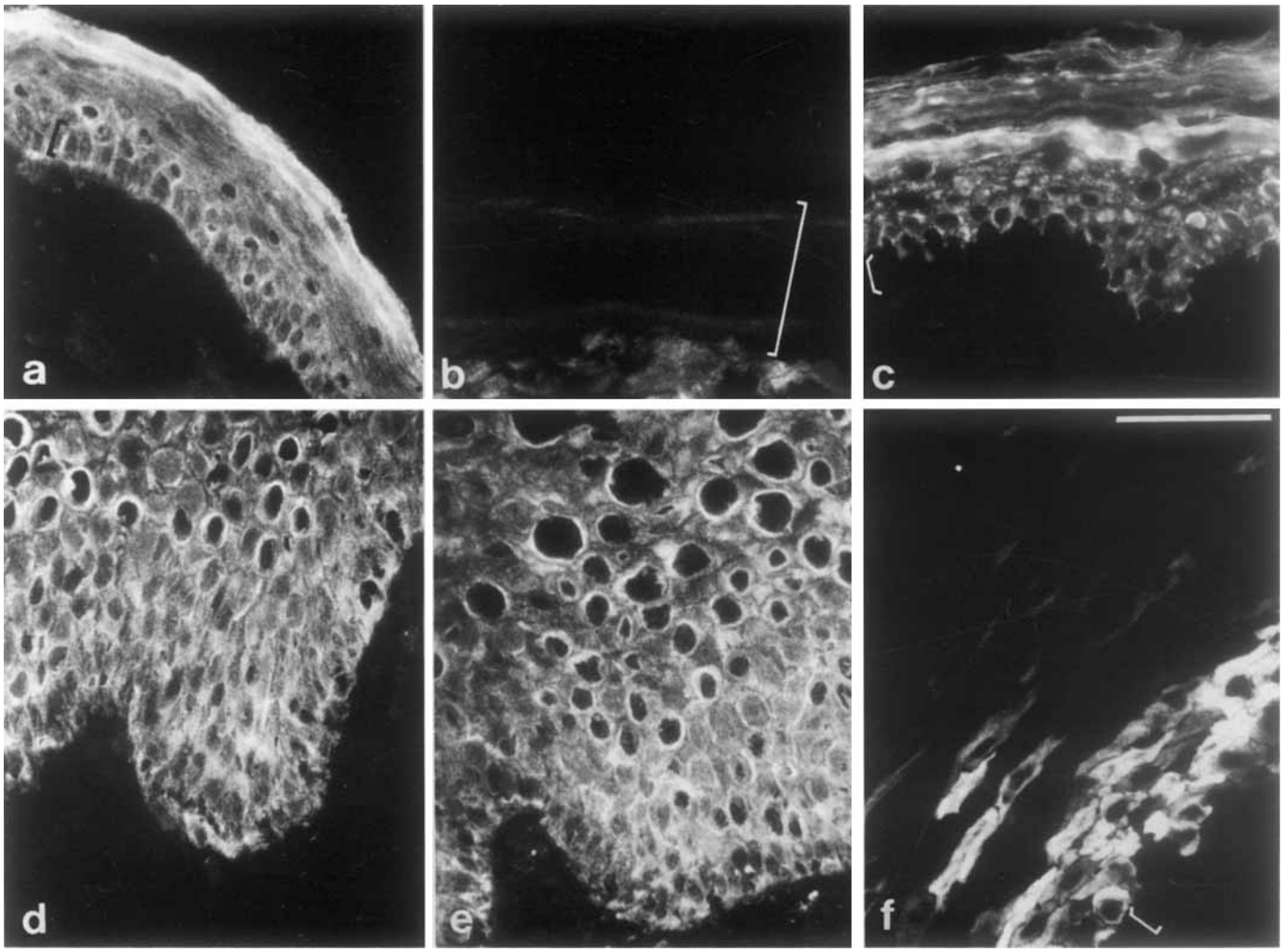


Fig. 4a–f. Immunofluorescence microscopy of sections of frozen human skin (**a–c**) and exocervix (**d–f**), with monoclonal antibodies K_G 8.13 (**a, d**), K_S 8.12 (**b, e**), and K_K 8.60 (**c, f**). K_G 8.13 stains all layers of both stratified squamous epithelia (**a, d**). K_S 8.12 is negative on the cornified stratified epithelium of the skin (**b**) and is uniformly positive on the noncornified stratified squamous epithelium of the exocervix (**e**). K_K 8.60 stains practically all cells of the suprabasal layers of the stratified keratinizing squamous epithelium of the epidermis. In the exocervix, K_K 8.60 nonuniformly labels only some of the cells present in suprabasal layers. The *brackets* in **a, c,** and **f** indicate the basal cell layer; in **b** the *bracket* indicates the entire epidermis. *Bar*, 50 μm

relationship between an epitope(s) in the two epidermal polypeptides that belong to different cytokeratin subfamilies (see below). Further examination of a variety of tissues covering the entire spectrum of cytokeratins did not reveal reactivity with any other polypeptides.

In Fig. 3, we summarize schematically the defined specificities of the three monoclonal antibodies used in the present study.

Immunofluorescence microscopy of specific cytokeratin polypeptides in normal human tissues

The staining of cornified (skin) and noncornified (exocervix) stratified squamous epithelia produced different patterns of labeling with the three monoclonal antibodies (Fig. 4). While K_G 8.13 labeled all epithelial cell layers of both tissues (Fig. 4a, d), K_S 8.12 was essentially negative on skin but produced extensive, rather uniform labeling of the exocervix (Fig. 4b, e). Several other stratified epithelia were also positive with this antibody (not shown; cf. Table 1), although the intensity of the reaction often appeared to be higher in the suprabasal cell layers. Antibody K_K 8.60 stained all suprabasal layers of the skin (Fig. 4c) and produced nonuniform labeling of some cells of the suprabasal layers of the exocervix (Fig. 4f). Positive labeling was observed either in single cells scattered throughout the tissue or in cell groups of various sizes. Various other noncornified stratified squamous epithelia, e.g., esophagus, were mostly negative (see Table 1).

Pseudostratified respiratory epithelium was uniformly positive for antibody K_G 8.13 (Fig. 5a), while only certain cells of the basal layer were stained with K_S 8.12 (Fig. 5b). None of the epithelial elements of the bronchi were stained with K_K 8.60 (Fig. 5c). Transitional epithelium of the urinary bladder and urethra was also extensively labeled with both K_G 8.13 and K_S 8.12 (Fig. 5d, e) but exhibited no detectable labeling with K_K 8.60 (Fig. 5f). In some sections, we gained the impression that certain individual luminal cells ('umbrella cells') did not react well with K_S 8.12, but this may have been due to the notorious problem of lowered

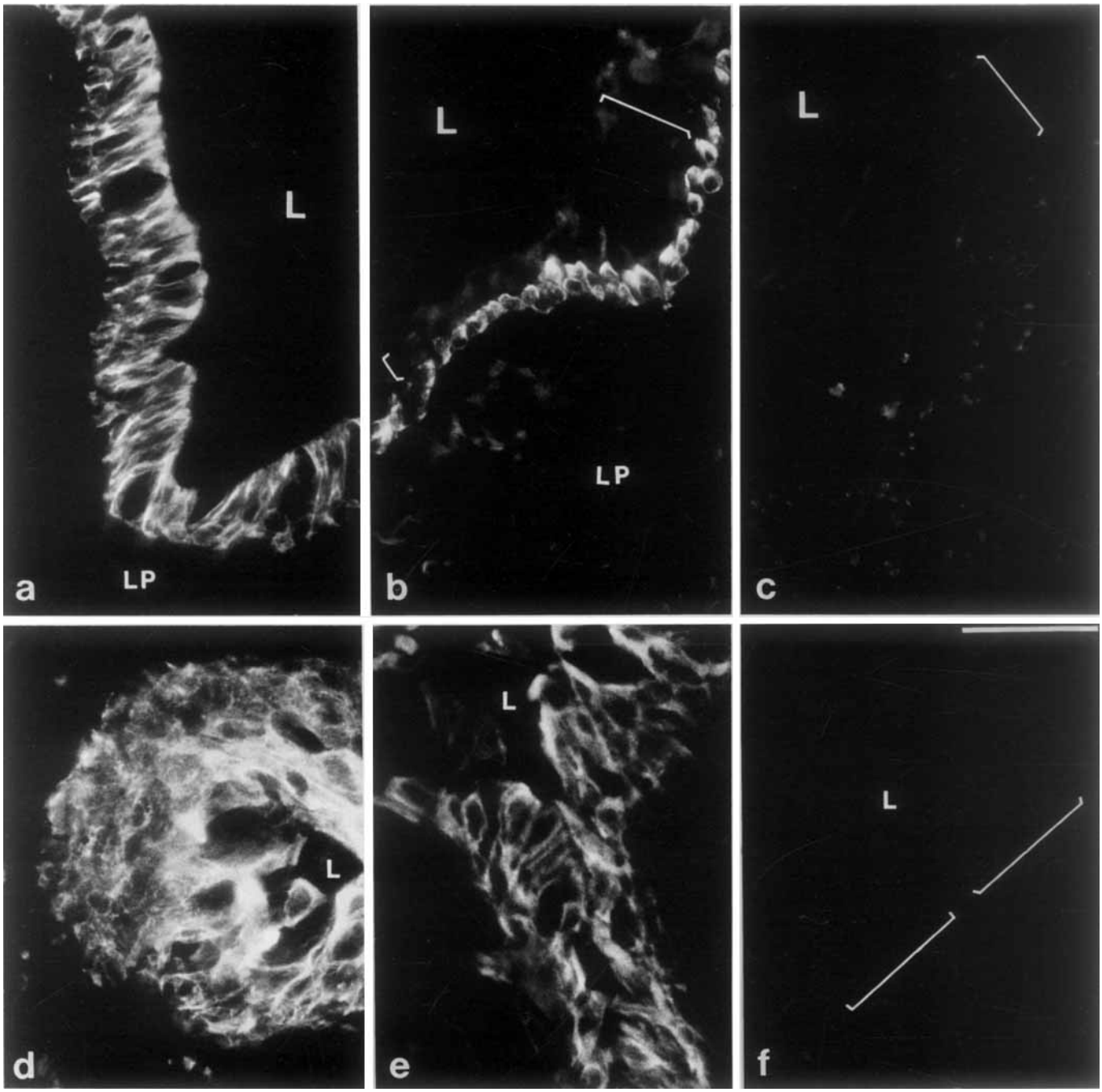


Fig. 5a–f. Immunofluorescence microscopy with monoclonal antibodies K_G 8.13 (**a, d**), K_S 8.12 (**b, e**), and K_K 8.60 (**c, f**) of frozen sections of a pseudostratified epithelium, i.e., respiratory tract mucosa (**a–c**) and the transitional epithelium of the urothelium (**d–f**). K_G 8.13 is uniformly positive on all epithelial cells (**a, d**). K_S 8.12 stains selectively only certain basal cells in the bronchial mucosa, whereas the goblet cells and columnar ciliated cells are negative (**b**). The urothelium appears to be uniformly positive with this antibody (**e**). K_K 8.60 does not stain the epithelial elements of both tissues. The *brackets* in **b** mark the location of unstained columnar cells; the *brackets* in **c** mark the location of the respiratory mucosa. The *brackets* in **f** indicate unstained urothelium. *L*, lumen; *LP*, lamina propria. *Bar*, 50 μm

antigen accessibility in these cells (cf. [13]). All of the simple epithelia examined were positively labeled with K_G 8.13 only, and they exhibited no significant labeling with the other two antibodies (Fig. 6; Table 1). None of the antibodies stained non-epithelial cells.

A summary of the specific reactivities of the different antibodies used with various normal human tissues is shown in Table 1.

Immunofluorescence localization of specific cytokeratin polypeptides in human carcinomas

A large variety of human tumors, mostly carcinomas, were examined using the three monoclonal antibodies. All squamous cell carcinomas tested were positively labeled with both K_G 8.13 and K_S 8.12 (Fig. 7; Table 2). Remarkably, some heterogeneity in the pattern of labeling with K_S 8.12

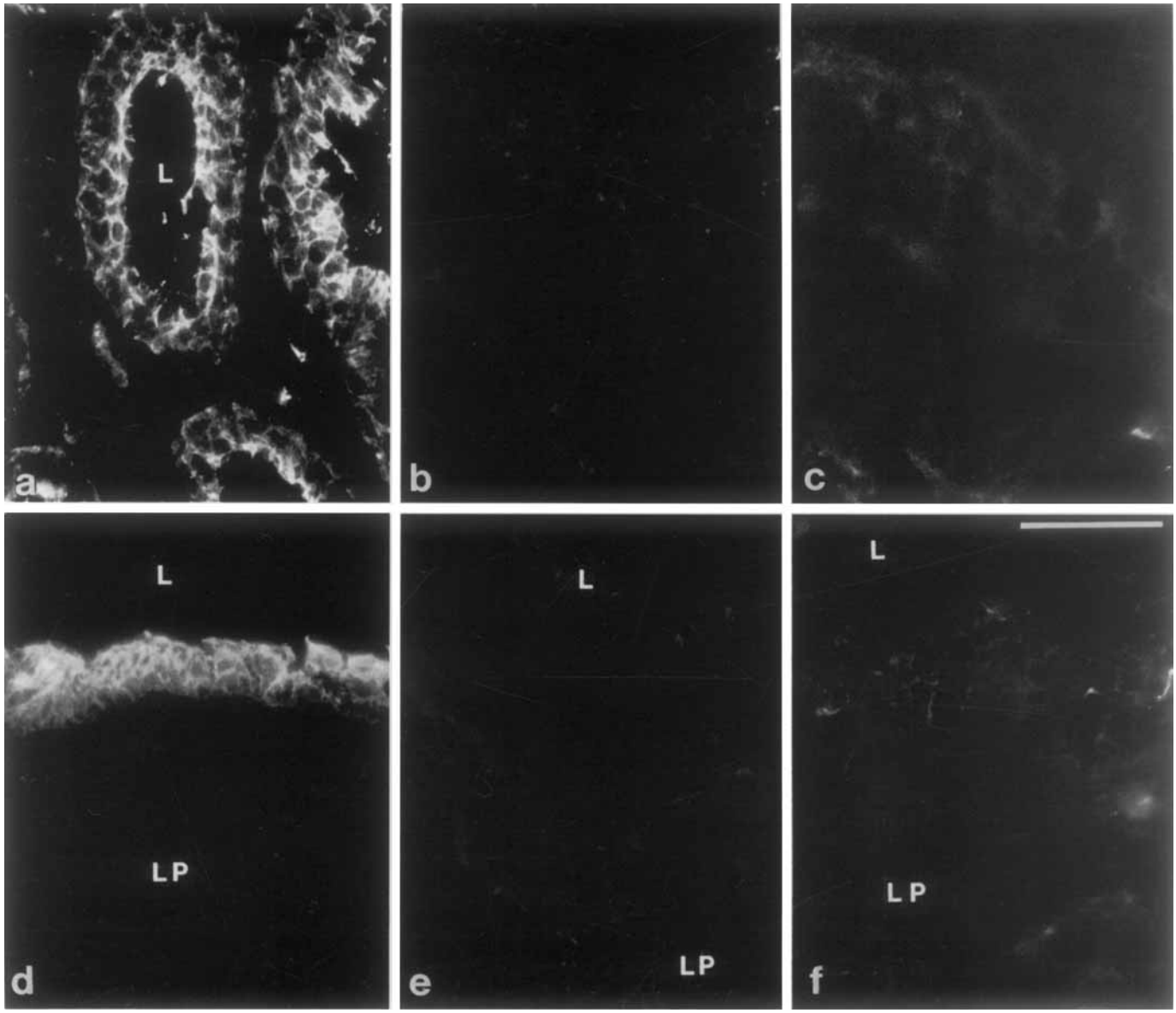


Fig. 6a–f. Immunofluorescence microscopy of simple epithelia, including crypts of the large intestine (**a–c**) and endocervical mucosa (**d–f**), with monoclonal antibodies K_G 8.13 (**a, d**), K_S 8.12 (**b, e**), and K_K 8.60 (**c, f**). Note the positive reaction with only the broadly cross-reacting antibody K_G 8.13. *LP*, lamina propria; *L*, lumen. *Bar*, 50 μm

was noted in certain tumors. In squamous cell carcinomas of the lung and cervix, the vast majority of cells were stained, with occasional unstained cells being located mostly in peripheral regions (Fig. 7). Other squamous cell carcinomas exhibited more 'patchy' reactivity with K_S 8.12. It should be pointed out that positive labeling with K_S 8.12 was also observed in squamous cell carcinomas derived from tissues which are normally not labeled with this antibody (e.g., epidermis; see Table 2).

Antibody K_K 8.60 was usually found to stain well or moderately differentiated squamous cell carcinomas in a rather heterogeneous fashion (typical examples are presented in Fig. 8). In some tumors, only sparsely distributed cells within the tumor mass were positive, whereas in others, the labeling was rather extensive. Often, relatively well circumscribed clusters of positively labeled cells were noted. In well-differentiated squamous carcinomas and in well-differentiated regions within squamous carcinomas, positive labeling was usually noted in cells around centers of cornification (Fig. 8d). As shown in Table 2, there was a general correlation between the level of morphological differentiation and the extent of labeling with K_K 8.60, probably reflecting the amount of cells expressing polypeptides nos. 10 and 11. All of the adenocarcinomas examined (over 30 tumors derived from a large variety of tissues; Table 2) exhibited positive staining with K_G 8.13 only, and they did not show any detectable reaction with K_S 8.12 and K_K 8.60 (Fig. 9).

The differential immunohistochemical labeling of adenocarcinomas and squamous carcinomas by K_S 8.12 and K_K 8.60 was used for the identification not only of primary tumors, but also of metastases in lymph nodes or other organs. As demonstrated in Fig. 10, lymph-node metastases of several squamous cell carcinomas were positively labeled

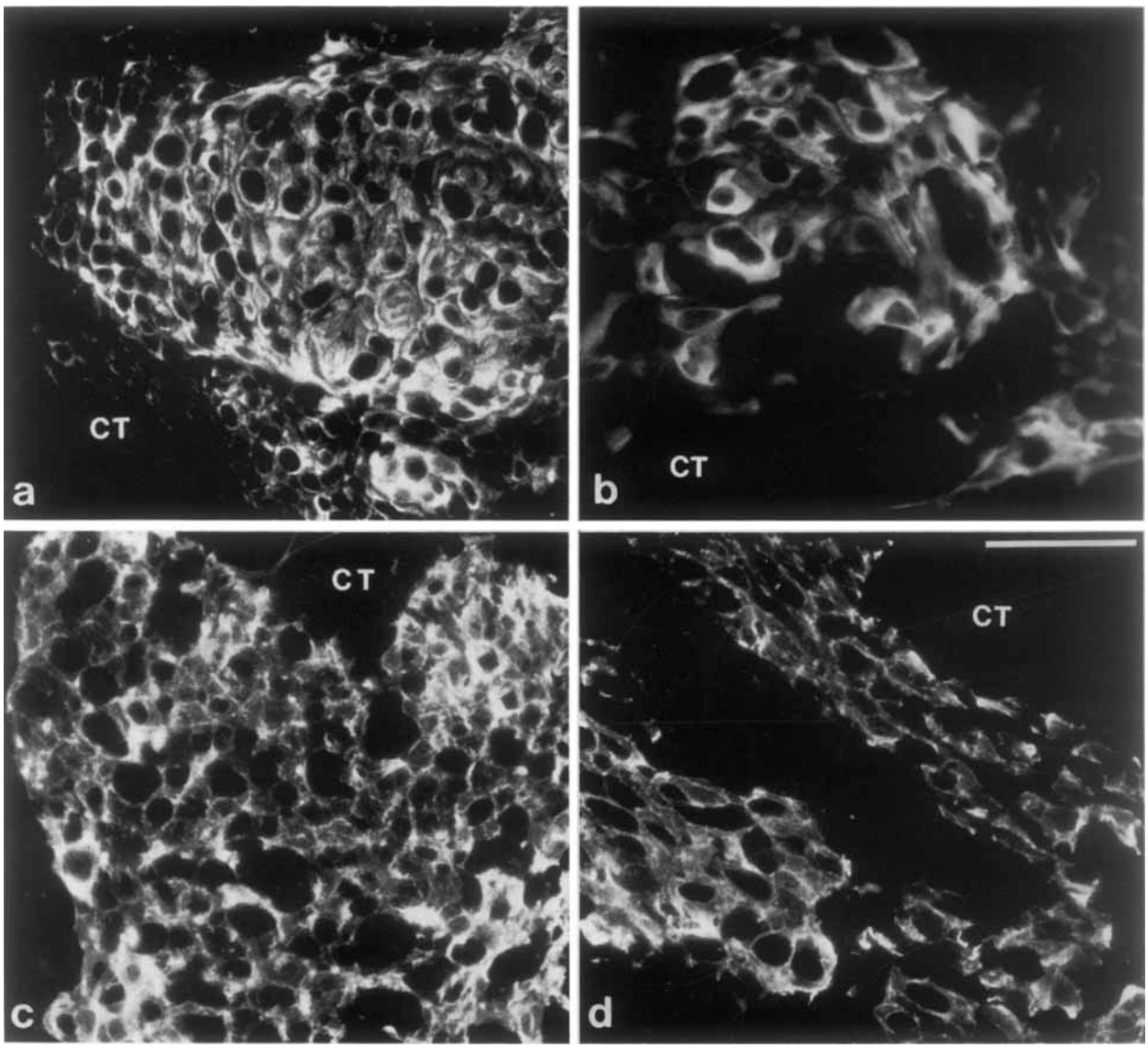


Fig. 7a–d. Immunofluorescence microscopy (frozen sections) of squamous cell carcinomas of the lung (**a, b**) and cervix (**c, d**) using monoclonal antibodies K_G 8.13 (**a, c**) and K_S 8.12 (**b, d**). Note the extensive cytoplasmic staining of neoplastic cells with both antibodies. *CT*, connective tissue of the stroma. *Bar*, 50 μm

with K_G 8.13 and K_S 8.12, and in some of these staining of sparse individual cells with K_K 8.60 was also observed. The patterns of labeling were similar to those seen in the respective primary tumors. Metastases of adenocarcinomas were not labeled by antibodies K_S 8.12 and K_K 8.60 (Fig. 10e, f).

Discussion

In recent years, IFs (particularly their organization and cell-type-restricted expression) have received a great deal of attention from cell and developmental biologists as well as from pathologists. In particular, antibodies specific to the various families of IFs are now widely used for studies of cell differentiation and the development of tissues, as well as for the diagnosis of tumors, notably those with a poorly defined morphology (for references, see Introduction). Of particular interest among the diverse IF proteins is the cytokeratin family, which forms the IFs of epithelial tissues. Considerable progress has recently been made with regard to the biochemical and immunochemical characterization of the diverse cytokeratin polypeptides and the complexes they form with each other [8, 11, 15, 20–24, 40, 44–47, 49]. The various cytokeratin polypeptides, i.e., 19 in human epithelia, can be grouped into two major subfamilies which share only relatively little sequence homology (<30%; cf [21, 45, 46]): the relatively 'basic' polypeptides (also known as type-B or type-II cytokeratins) and the 'acidic' polypeptides (type A or type I). It has also been shown that the formation of cytokeratin filaments requires the presence of at least one polypeptide from either subfamily which form typical heterotypic tetramer complexes and finally assemble into IFs (e.g. [39, 46]).

Of great importance in studies of the development of normal epithelia as well as in the cell typing of epithelial tumors is the availability of well-defined antibody reagents. The monoclonal antibodies which have as yet, been adequately characterized can be grouped into three major categories:

Table 2. Immunohistochemical staining of human tumors with monoclonal antibodies to different cytokeratin polypeptides

Tumors	Number of cases	Antibody			Polypeptides present[e]
		K_G 8.13	K_S 8.12	K_K 8.60	
Squamous cell carcinomas					
Lung: well differentiated	3	+	+	+NU	ND
Lung: moderately to poorly differentiated	5	+	+	–	4[c], 5, 6, 8, (13), (14)[c], (15)[c], 17, (18), 19
Anorectum: poorly differentiated	1	+	+[a]	–	5, 6, (8), 14, 16, 17, (18), 19
Esophagus: moderately differentiated	2	+	+[d]	+NU	4[c], 5[c], 6, (8), (10/11), 14, (16), 17, (19)
Tongue: well differentiated	1	+	+[a]	+NU	(5), 6, (10/11), 14, 16, 17
Cervix: moderately to poorly differentiated	6	+	+	±[b]NU	5, 6, 7[c], (8), 13, 14[c], 15, (16), 17, (18), 19
Skin: well differentiated	2	+	+[a]	+NU	(1), 4[c], 5, 6, (10/11), 14, 16, 17
Larynx: well differentiated	1	+	+	+NU	ND
Adenocarcinomas					
Stomach	2	+	–	–	7[c], 8, (18), 19
Breast	7	+	–	–	7, 8, 18, 19
Pancreas	1	+	–	–	7, 8, (17), (18), 19
Lung	7	+	–	–	7, 8, 18, 19
Colon	4	+	–	–	8, 18, 19
Endometrium	1	+	–	–	ND
Esophagus	1	+	–	–	ND
Prostate	1	+	–	–	ND
Basal cell epithelioma	5	+	–	–	5, (8), 14, (15), 17
Mesothelioma of pleura: epithelial type	1	+	–	–	5, 8, (14), 18, 19

NU, nonuniform labeling; ND, not determined

[a] Most tumor cells positive
[b] Three cases negative and three cases nonuniformly positive
[c] Not present in all cases
[d] In one case, most tumor cells were positive; in the other case, less than 50% of the tumor cells were positive
[e] Determined by two-dimensional gel electrophoresis [6, 29, 31, 32, 39]. Numbers in parenthesis indicate polypeptides present in minor amounts

1. Broadly cross-reacting antibodies which interact with epitopes present on most, if not all cytokeratin polypeptides of one or both subfamilies. An example is antibody K_G 8.13 which reacts with several cytokeratin polypeptides of the basic type-II subfamily as well as with the type-I polypeptide no. 18 [19].

2. Antibodies whose specificity is restricted to some or all members of the same subfamily. Examples in this category are the monoclonal antibodies AE1 and AE3 described by Sun and coworkers [8, 11, 47, 49].

3. Polypeptide-specific antibodies which are directed against a single polypeptide or a small group of related polypeptides. Examples of such antibodies are those of the CK 1–4 antibody series described by Debus et al. (see [10]) and antibody RGE-53 described by Ramaekers et al. [41], all of which react specifically with cytokeratin no. 18.

The two new antibodies described in the present study, namely K_S 8.12 and K_K 8.60, which both react with polypeptides of the acidic (type I) subfamily, fall into the last class. Antibody K_S 8.12 reacts specifically with polypeptides nos. 13 and 16. It is similar, but not identical, to the cytokeratin-no.-13-specific antibodies 1C7 and 2D7 that have recently been described by van Muijen et al. [50]. Its pattern of reactivity closely corresponds to the patterns of synthesis of cytokeratins nos. 13 and 16 as determined by gel electrophoresis [29–32]. Whether the staining of certain basal cells

Fig. 8a–d. Immunofluorescence microscopy of squamous cell carcinomas of the lung using monoclonal antibody K_K 8.60, showing various patterns of staining. **a** Positive staining of sparsely distributed individual cells; **b** tumor region showing extensive staining of most of the cells; **c** positive staining of a distinct cluster of cells; **d** positive staining of cornification foci. *HP*, 'horn pearls'. Note that the stroma is not stained. *Bar*, 50 μm

Fig. 9a–c. Immunofluorescence microscopy of a colon adenocarcinoma using antibodies K_G 8.13 (**a**), K_S 8.12 (**b**), and K_K 8.60 (**c**). Note the positive staining of tumor cells with only K_G 8.13. *L*, lumen. *Bar*, 50 μm

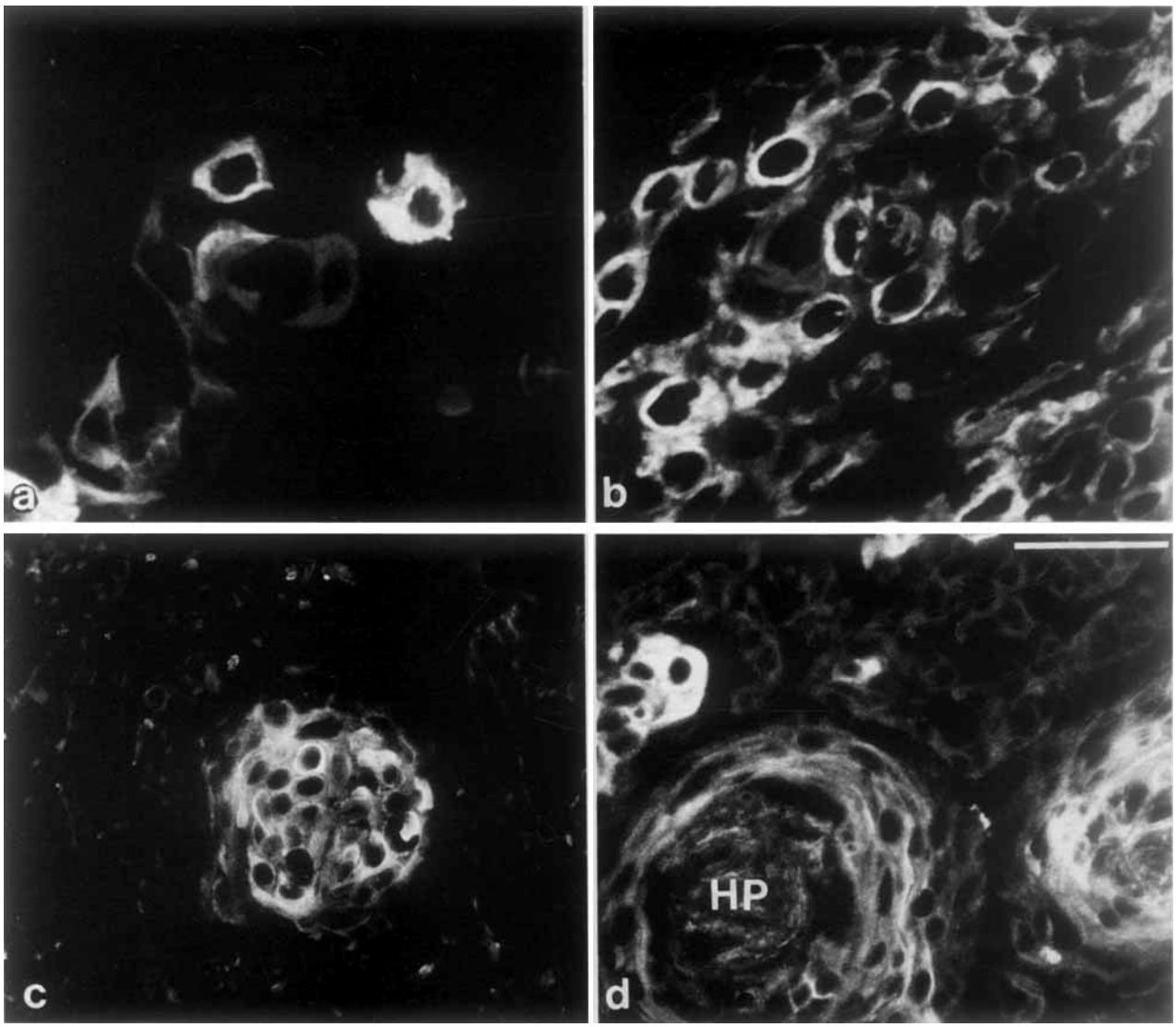


Fig. 8a–d

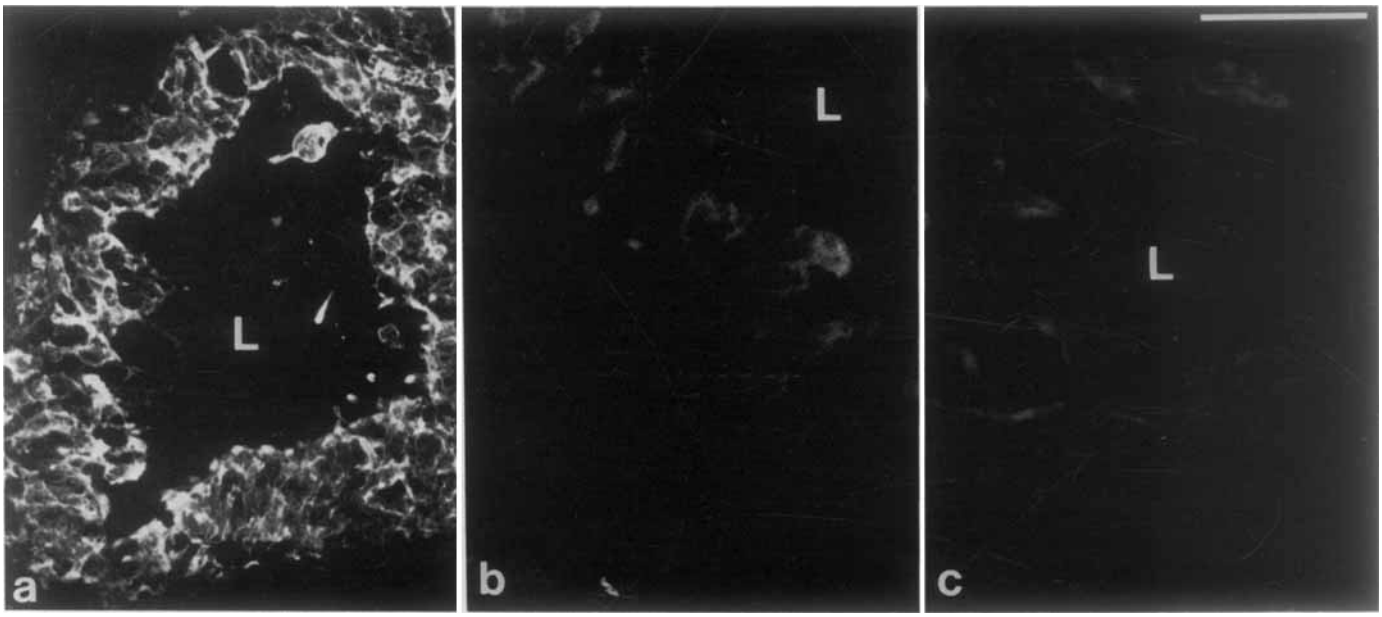


Fig. 9a–c

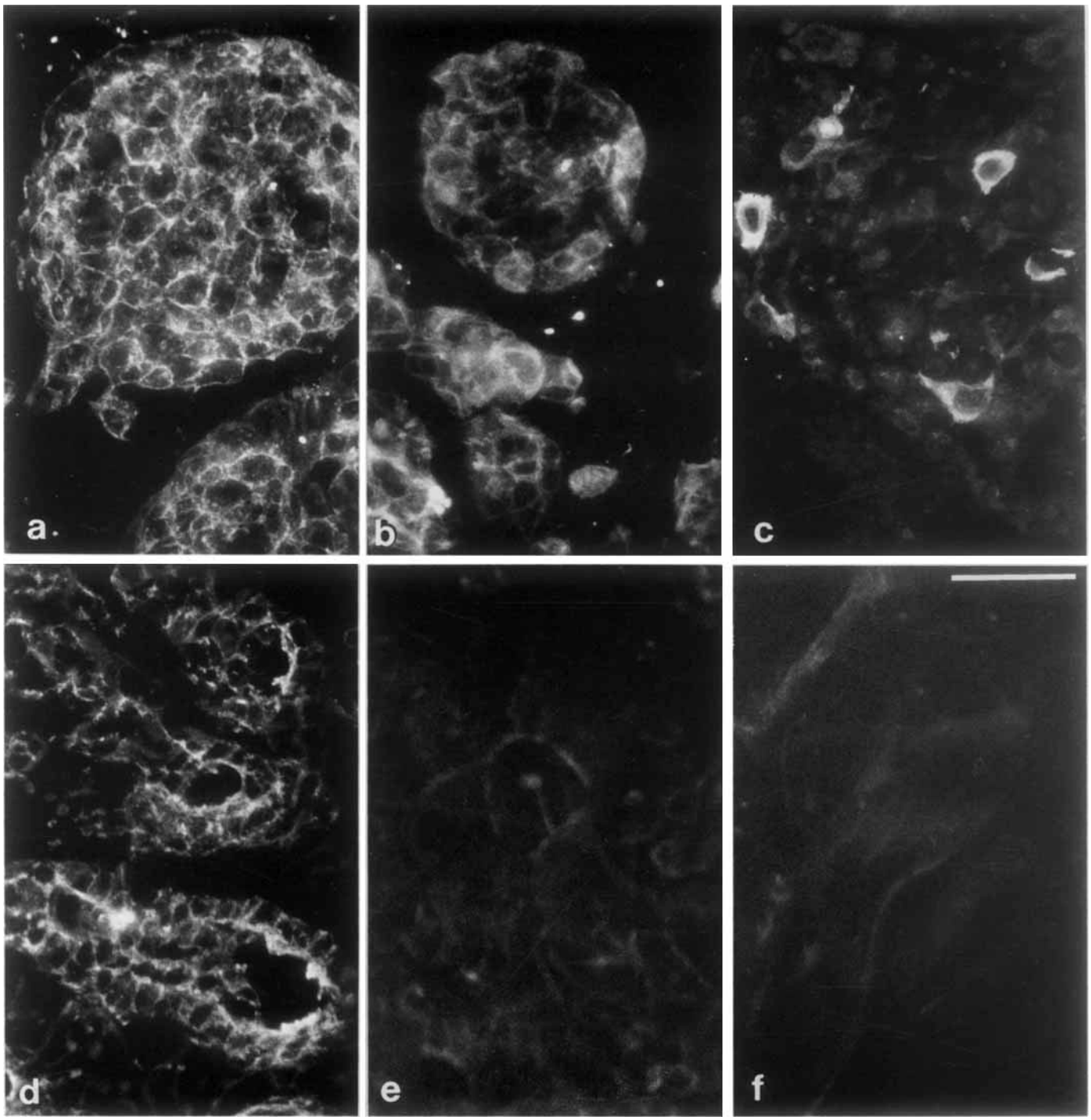


Fig. 10a–f. Immunofluorescence microscopy of lymph-node metastases of a squamous cell carcinoma of the lung (**a–c**) and an adenocarcinoma of the prostate gland (**d–f**) with monoclonal antibodies K_G 8.13 (**a, d**), K_S 8.12 (**b, e**), and K_K 8.60 (**c, f**). Antibody K_G 8.13 stained both the squamous carcinoma and the adenocarcinoma, whereas K_S 8.12 stained selectively only the squamous cell carcinoma. Antibody K_K 8.60 positively stained only a few cells within the squamous cell carcinoma mass. *Bar*, 50 μm

of esophagus, tongue, vagina, and exocervix by antibody K_S 8.12 in our study but not by antibodies 1C7 and 2D7 of van Muijen et al. [50] reflects the presence of cytokeratin no. 16 in these cells or is due to selective masking of the epitope(s) recognized by antibodies 1C7 and 2D7 remains to be examined. Antibody K_K 8.60 reacts predominantly, if not exclusively, with polypeptides nos. 10 and 11, and thus appears to react in a fashion similar to the cytokeratin-no.-10/11 antiserum described by Jorcano et al. [24]. While K_S 8.12 reacts with noncornified stratified epithelia, e.g., the mucosae of the esophagus, tongue, and exocervix, the expression of polypeptides nos. 10 and/or 11 is prominent only in certain cornified epithelia such as the epidermis. However, individual cells positive for polypeptides nos. 10 and/or 11 were also seen in suprabasal layers of some 'non-cornified' epithelia. The transitional epithelium of the uri-

nary system, which has been shown to contain polypeptide no. 13 [29, 39, 50], was also positive with K_S 8.12. Interestingly, in the pseudostratified epithelium of the respiratory tract, antibody K_S 8.12 differentially stained only certain basal cells in a fashion resembling the reactivity of a cytokeratin antiserum recently described by Blobel et al. [6].

Our results also point to an apparent antigenic cross-reactivity between cytokeratins nos. 13 and 16 on the one hand, as well as between polypeptides nos. 10 and 11 on the other. These findings suggest that, in addition to subfamily-specific epitopes common to all four polypeptides, i.e., nos. 10, 11, 13 and 16, which react with antibodies such as AE1 [8], there are other epitopes that appear to be shared by only a small subset of polypeptides within the acidic subfamily (this study) or are present in only one polypeptide (e.g. [50]). It is to be expected that increasing knowledge of the amino acid sequences of the various polypeptides will shed light on the structural basis of these antigenic cross-reactivities.

Obviously, antibodies K_S 8.12 and K_K 8.60 are valuable probes for studies of the normal development of stratified epithelia in embryogenesis, as well as for studies of pathological processes such as squamous metaplasia. In addition, they represent important complementary antibody reagents for tumor diagnosis. The striking feature of antibody K_S 8.12 is its specific staining of cells present in many (perhaps all) squamous carcinomas regardless of their particular histogenic origin, whereas in contrast, adenocarcinomas are invariably negative for K_S 8.12. Hence, this antibody should be most useful for distinguishing between these two types of tumors, both at their primary locations and at their metastatic sites. Comparison of the immunohistochemical data with the biochemical analyses suggests that the staining of cells of squamous carcinomas of lung and cervix is predominantly due to the presence of cytokeratin no. 13, whereas in other squamous cell carcinomas, cytokeratin no. 16 may be responsible (Table 2). In several squamous cell carcinomas, a variable but usually minor proportion of tumor cells was not stained by K_S 8.12, often resulting in a patchy pattern of staining. Whether these differences in the reactivity of different cells of the same tumor – as also noted using other monoclonal antibodies such as antibodies CK1–4 against cytokeratin no. 18 [10] or specific antibodies against cytokeratins nos. 4 and 13 [50] – indicate true differences of gene expression or are due to local differences of epitope exposure (i.e., masking) cannot be decided at present.

Remarkably, both biochemical determinations and the immunohistochemical labeling obtained with K_S 8.12 suggest that polypeptides nos. 13 and/or 16 are not always present in significant amounts in the normal tissues from which squamous carcinomas have evolved. For example, epidermis is completely negative for K_S 8.12, whereas primary squamous cell carcinomas of the skin are often strongly positive. This may be seen as evidence for the concept that polypeptide no. 16 is absent from normal epidermis (see also [29]) but occurs in hyperproliferative states of epidermis as well as in the outer root sheaths of hair follicles [8, 11, 29, 47, 52]. Moreover, we have noticed that cells of the simple epithelium of the endocervix that take a route of differentiation toward stratification and form squamous metaplasia begin to react positively with antibody K_S 8.12 (B. Czernobilsky, O. Leitner-Gigi, R. Levy, and B. Geiger, manuscript in preparation).

Antibody K_K 8.60 against cytokeratins nos. 10 and 11 seem to be a good marker for terminal differentiation in the epidermis, as it stains only suprabasal cell layers in human skin. In this respect, antibody K_K 8.60 exhibits a pattern of reactivity similar to that previously described for a guinea-pig antiserum specific for cytokeratin polypeptides 10 and 11 [24], thus confirming the intraepidermal localization of these proteins proposed by Sun et al. (for reviews, see [8, 47, 52]). Antibody K_K 8.60 is also important in that it allows the detection of cytokeratin-10/11-expressing cells in thymus, vagina, and exocervix; previously, this was only possible by gel electrophoresis of cytoskeletal proteins from tissue sections [31, 32, 39]. Bearing in mind the high resolution power of immunofluorescence microscopy, this antibody should be of great value in studies of terminal differentiation processes in diverse epithelia, as well as for the detection of minor cell populations exhibiting a different kind (or degree) of differentiation that may escape detection by other methods. One example of this is our finding of some cytokeratin-13/16 positivity in basal cells of the bronchial epithelium, which explains the findings of Blobel et al. [6] that these cells stain selectively for certain cytokeratins characteristic of stratified epithelia; it also explains why Blobel et al. were unable to detect such cytokeratins by gel electrophoresis of tissue samples, as this procedure is not sensitive enough to identify cytokeratins present in only a minor proportion of the cells of the tissue.

A nonuniform pattern of labeling with antibody K_K 8.60 was also detected in many squamous cell carcinomas. This observation indicates that cells present in the same tumor can, in spite of their probable clonal nature and their generally common environment, display remarkable differences in their expression of major cytoskeletal proteins. As already mentioned with regard to normal tissues, we cannot decide at present whether these differences of staining with the cytokeratin-no.-10/11-specific antibody reflect differences of synthesis or selective masking of the epitope. Similar cell heterogeneity has previously been reported with respect to metastatic potential and drug sensitivity [12, 33]. This variability in the expression of specific cytokeratins in cells in the same region of the same tumor may be due to local variations in the tumor cell microenvironment. However, it may also represent the existence of subpopulations embarked on different pathways of squamous differentiation.

All in all, for a given carcinoma, there seems to be a general correlation between the extent of morphologically defined squamous differentiation and the expression of cytokeratin polypeptides 10 and/or 11. As already mentioned, well-differentiated squamous cell carcinomas with many foci of cornification usually contain cells stained by K_K 8.60, whereas poorly differentiated carcinomas are hardly labeled or completely unlabeled. Since staining with K_K 8.60 is apparently more sensitive than conventional histological staining for the detection of early stages of cornification, this antibody may be a valuable immunocytochemical tool for defining the state of differentiation of a given squamous carcinoma.

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