

Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody

Orith Gigi, Benjamin Geiger*, Zelig Eshhar, Roland Moll¹, Erika Schmid¹, Stefanie Winter¹, Dorothea L.Schiller¹, and Werner W.Franke¹

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel, and ¹Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, FRG

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A monoclonal antibody derived from a mouse immunized with bovine epidermal prekeratin has been characterized by its binding to cytoskeletal polypeptides separated by one- or two-dimensional gel electrophoresis and by immunofluorescence microscopy. This antibody (K_G 8.13) binds to a determinant present in a large number of human cytokeratin polypeptides, notably some polypeptides (Nos. 1, 5, 6, 7, and 8) of the 'basic cytokeratin subfamily' defined by peptide mapping, as well as a few acidic cytokeratins such as the epidermis-specific cytokeratins Nos. 10 and 11 and the more widespread cytokeratin No. 18. This antibody reacts specifically with a wide variety of epithelial tissues and cultured epithelial cells, in agreement with previous findings that at least one polypeptide of the basic cytokeratin subfamily is present in all normal and neoplastic epithelial cells so far examined. The antibody also reacts with corresponding cytokeratin polypeptides in a broad range of species including man, cow, chick, and amphibia but shows only limited reactivity with only a few rodent cytokeratins. The value of this broad-range monoclonal antibody, which apparently recognizes a stable cytokeratin determinant ubiquitous in human epithelia, for the immunohistochemical identification of epithelia and carcinomas is discussed.

Key words: cytoskeleton/intermediate filaments/keratins/epithelial differentiation/histodiagnosis

Introduction

The diverse classes of intermediate-sized filaments ('intermediate filaments') share common principles of filament ultrastructure and molecular arrangement (Steinert *et al.*, 1978; Lazarides, 1980, 1982; Anderton, 1981; Franke *et al.*, 1982b; Holtzer *et al.*, 1982; Osborn *et al.*, 1982) and also show partial sequence homology of their constituent polypeptides (Geisler *et al.*, 1982a, 1982b). On the other hand, different subclasses of intermediate filaments can be distinguished, using biochemical and immunological techniques, by their polypeptide constituents, which appear to be expressed in specific patterns related to cell differentiation (Bennett *et al.*, 1978; Franke *et al.*, 1978a, 1979a, 1982b; Schmid *et al.*, 1979; Sun *et al.*, 1979; Holtzer *et al.*, 1982; Osborn *et al.*, 1982). One subclass of intermediate filaments, which is characteristic of epithelial cells, contains a family of many related but not identical polypeptides ('cytokeratins') (Franke *et al.*, 1978a, 1978b, 1979a, 1982a; Sun and Green, 1978; Sun *et al.*, 1979). Up to 19 different polypeptides which are ex-

pressed in different patterns in different epithelia have so far been distinguished in human cells (e.g., Doran *et al.*, 1980; Winter *et al.*, 1980; Franke *et al.*, 1981a, 1981b, 1981c, 1982b, Fuchs and Green, 1981; Wu and Rheinwald, 1981; for review, see Moll *et al.*, 1982a). This diversity, however, presents a problem in the immunological identification of proteins of this type in epithelial tissues and cultured cells because the specific antibodies used cannot be assumed *a priori* to react with each of the specific cytokeratin polypeptides of a given cell. Indeed, immunofluorescence microscopic studies have presented examples showing that antibodies raised against cytokeratins of one tissue (i.e., human or bovine epidermis) react with some but not all epithelial tissues. For example, several of the antibody preparations previously described have not reacted with hepatocytes, pancreatic acinar cells, secretory cells of mammary gland, and most types of kidney epithelial cells (Franke *et al.*, 1978a, 1978b, 1979a, 1979b, 1980, 1981a; Sun *et al.*, 1979; Schlegel *et al.*, 1980a; Asch *et al.*, 1981; Banks-Schlegel *et al.*, 1981; Krepler *et al.*, 1981; Lane, 1982). The importance of specific, broad-spectrum cytokeratin antibodies has become apparent recently in view of the growing interest in the use of such antibodies for tumor diagnosis (Bannasch *et al.*, 1980; Battifora *et al.*, 1980; Schlegel *et al.*, 1980a, 1980b; Altmannsberger *et al.*, 1981, 1982; Caselitz *et al.*, 1981; Gabbiani *et al.*, 1981; for reviews, see Moll *et al.*, 1982a; Osborn *et al.*, 1982; Ramaekers *et al.*, 1982). It would thus be desirable to have well defined monoclonal antibodies which either recognize cytokeratin determinants present in all epithelia or show strict tissue specificity.

Here we describe a monoclonal antibody raised against a determinant of epidermal prekeratin from bovine muzzle which also reacts with a broad range of cytokeratin polypeptides from diverse tissues and species. Moreover, this broadly reacting antibody K_G 8.13 not only reveals an immunological relatedness between various cytokeratin polypeptides of the 'basic subfamily' but also shows that some other, more acidic cytokeratin polypeptides share some immunological similarity with polypeptides of this subfamily.

Results

Eighty four hybrid cultures from one fusion (mouse No. 8) and 22 from the second fusion (mouse No. 3) produced antibodies to keratins as revealed by solid phase radioimmunoassay. These antibodies were further analyzed for their activity toward various preparations and combinations of cytokeratin polypeptides from different organs and species. In addition, the various monoclonal antibodies were screened for their ability to decorate cytoskeletal elements of various tissues and cell lines using the immunofluorescence method. Antibodies produced by one of these clones, denoted K_G 8.13 (K, keratin; G, general; mouse No. 8; clone No. 13), was found to stain all human epithelial tissues examined and was therefore characterized in detail. The antibodies produced by the cultured hybridoma cells belong to the IgG₂ class. Two-dimensional gel electrophoresis of these antibodies [purified from culture medium, supplemented with IgG-free horse

*To whom reprint requests should be sent.

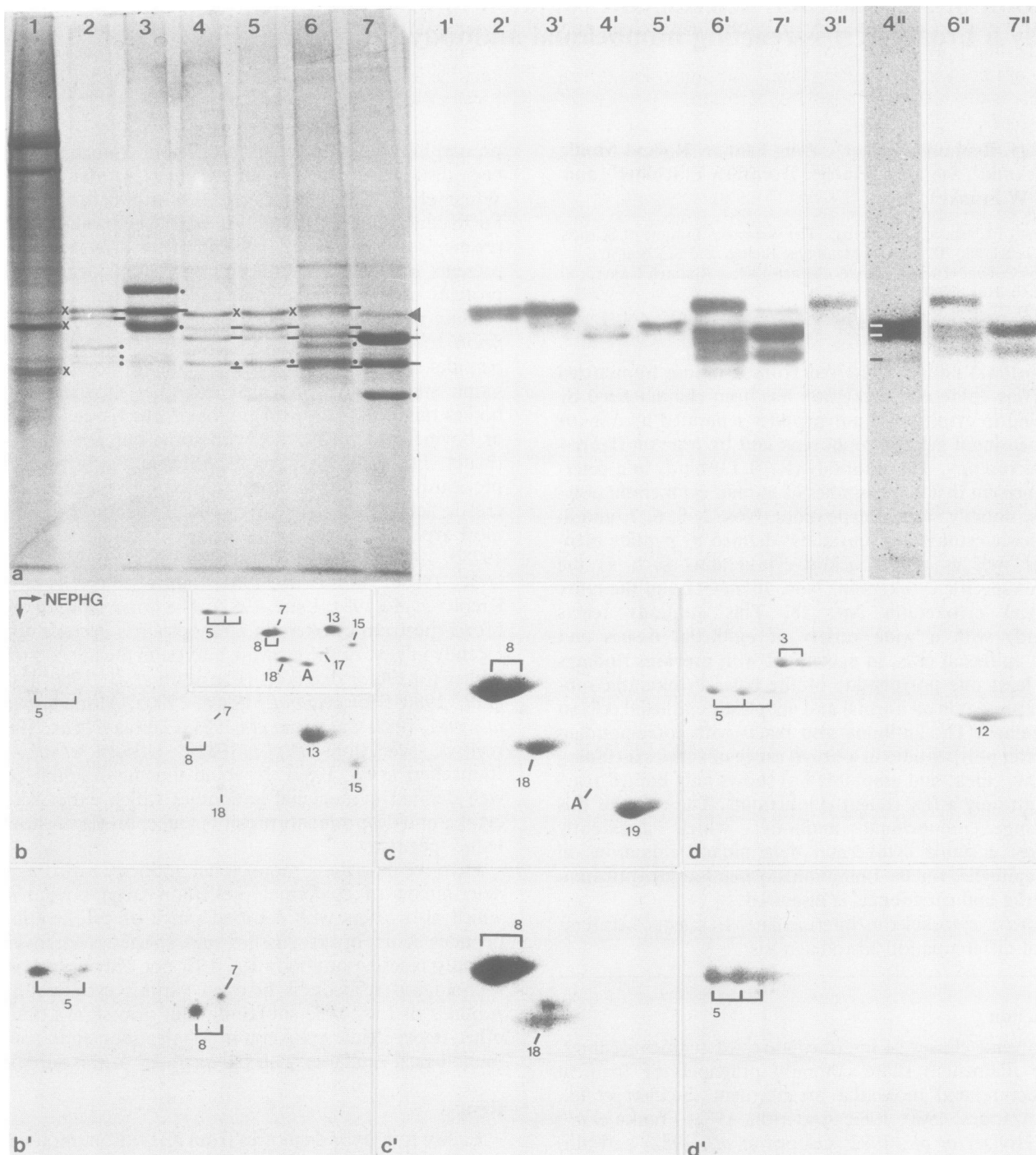


Fig. 1. One- (a) and two-dimensional (b–d) immunoblots using antibody K_G 8.13 on diverse cytoskeletal preparations from human cells and tissues. Symbols in a: Bars, cyokeratin polypeptides showing positive reaction with antibody K_G 8.13; dots, cyokeratin polypeptides which do not react; x, some cytoskeletal proteins other than keratins (from top to bottom in slot 1: vimentin, desmin, actin). (a) Coomassie blue stained gel (slots 1–7) and corresponding autoradiographs of immunoblot experiment using a parallel gel (slots 1'–7' and slots 3''–7'' present different exposure times). Slot 1, myometrium; slot 2, plucked hair follicles (from top to bottom: cyokeratin polypeptides Nos. 5, 6, 14, 16, 17); slot 3, cornea (polypeptides Nos. 3, 5, 12); slot 4, HeLa cells (cyokeratins Nos. 7, 8, 17, 18); slot 5, Henle-407 cell culture (HeLa pattern; same bands as in 4 but with different intensities of polypeptides Nos. 7 and 8); slot 6, A-431 carcinoma cells (cyokeratins Nos. 5, 7, 8, 13 which co-migrates with No. 8, 15, 17, 18); slot 7, MCF-7 cell culture (cyokeratins Nos. 8, 18, 19). Note positive reactions of cyokeratins Nos. 5, 7, 8, and 18. Slot 3'', relatively short exposure of same gel as in 3' (the reaction below the positive band No. 5 probably represents degradation products; see also Figure 3b). Slot 4'', relatively long exposure of same gel as in Figure 1, 4', showing reaction of cyokeratin No. 18 of HeLa cells which is present only in minor amounts. Slots 6'' and 7'', short exposures of gels shown in slots 6' and 7', allowing resolution of individual cyokeratin bands. The triangle in slot 7 denotes an unidentified band which also shows a weak reaction with antibody K_G 8.13. (b–d) Two-dimensional gel electrophoresis (Coomassie blue staining) of cytoskeletal proteins from A-431 cells (b; insert, autoradiograph of [³⁵S]methionine labeled cytoskeletal proteins revealing minor components), MCF-7 cells (c), and corneal tissue (d). (b')–(d') Autoradiographs of immunoblots showing reaction of antibody K_G 8.13 on gels in parallel to b (b'), c (c'), and d (d'). Note reaction in A-431 cytoskeletons with cyokeratins Nos. 5, 7, 8, and 18 (b', component No. 18 reacts only very weakly), in MCF-cytoskeletons with cyokeratins Nos. 8 and 18 (c'), and in corneal cells only with cyokeratin 5 (d'). Designations as introduced for human cyokeratins by Moll *et al.* (1982a). A, actin.

serum by chromatography on protein A coupled to Sepharose CL-4B (Ey *et al.*, 1978)] revealed only one heavy chain and one light chain polypeptide (not shown).

When antibody K_G 8.13 was tested in immunoblot experiments on cytoskeletal polypeptides separated by gel electrophoresis from diverse human tissues and cultured cells, a specific reaction with certain cytokeratins was observed (Figures 1–3). The antibody did not react with other cytoskeletal proteins including actin, desmin, vimentin (e.g., Figure 1a), and glial filament of cultured glioma cells (not shown). However, antibody K_G 8.13 did not react with all cytokeratin polypeptides. Strong reaction was observed with cytokeratin polypeptides Nos. 5, 6, 7, and 8, which belong to the 'basic subfamily' as defined by peptide mapping (for designations see Moll *et al.*, 1982a; Schiller *et al.*, 1982). However, cross-reaction was also obtained with cytokeratin No. 18 (Figure 1a–c), a component which is clearly distinguished from the other four polypeptides by its peptide map (Schiller *et al.*, 1982). On the other hand, the neutral-to-basic cytokeratin polypeptide No. 3 of cornea did not react with this antibody (Figures 1a, slot 3, and 1d). When epidermal keratins were examined (Figure 2) the epidermis-specific cytokeratin No. 1 strongly bound this antibody, as expected from the similarity of its peptide map with those of other cytokeratins of the basic subfamily (Schiller *et al.*, 1982). In addition, this antibody reacted with some components which were slightly lower in mol. wt. than cytokeratin No. 1 and were probably proteolytically derived from it (Figure 2b; these components seem to be enriched in *stratum corneum*; cf., Fuchs and Green, 1978, 1979, 1980). Unexpectedly, epidermis-specific cytokeratins Nos. 10 and 11, which are acidic but share no apparent peptide map homology with other acidic cytokeratins such as polypeptides 9 and 13–19 (cf. Moll *et al.*, 1982a, 1982b; Schiller *et al.*, 1982), were also reactive with K_G 8.13 (Figure 2c). In other stratified epithelia such as cornea, tongue, and esophagus the basic components 5 and 6

were the only positive ones (Figures 1d and 3a,b), whereas cytokeratins Nos. 3 and 4 did not react. In cytoskeletons from certain simple epithelia such as intestinal mucosa, cytokeratins 8 and 18 were positive whereas the small acidic cytokeratin No. 19 was negative (Figure 3c). The reactivities of antibody K_G 8.13 on human cytokeratin polypeptides are

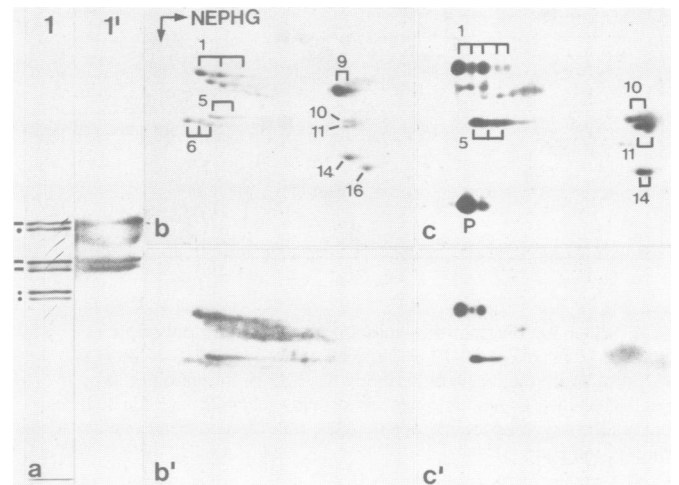


Fig. 2. Immunoblots using antibody K_G 8.13 on human epidermal prekeratins. (a, slot 1) SDS-PAGE of human foot sole epidermis microdissected from frozen sections (slot 1, Coomassie blue staining; bars and dots denote, from top to bottom, cytokeratins Nos. 1, 9, 5, 6, 14, 16). (a, slot 1') Autoradiograph of immunoblot of a, slot 1. (b) Two-dimensional gel electrophoresis (horizontal arrow, NEPHG electrophoresis) of material shown in (a); (b'), corresponding immunoblot. Note positive reaction with cytokeratins Nos. 1, 5, and 6 as well as with a series of polypeptides slightly smaller and more acidic than prekeratin No. 1 (b'; cf. a, slot 1'). (c) Two-dimensional gel electrophoresis (Coomassie blue staining) of prekeratins of female breast epidermis; (c') autoradiograph of corresponding immunoblot. Note strong reaction with components Nos. 1 and 5 and weaker reaction with components Nos. 10 and 11. P, phosphoglycerokinase added for reference.

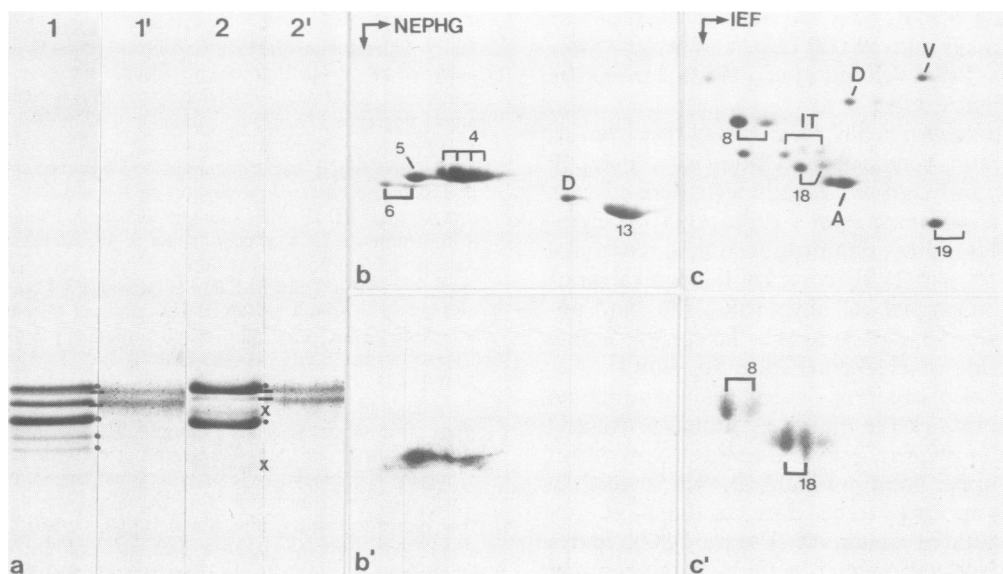


Fig. 3. Immunoblots using antibody K_G 8.13 on cytoskeletal preparations of human non-cornifying stratified squamous epithelia (a,b) and intestinal mucosa (c). (a, slot 1) SDS-PAGE of tongue cytokeratins (Nos. 4, 5, 6, 13, 14, 16; bars and dots as in Figure 2); slot 1', corresponding immunoblot; slot 2, SDS-PAGE of esophagus epithelium (cytokeratins Nos. 4, 5, 6, 13; upper x designates desmin from *muscularis mucosae*, lower x designates residual actin); slot 2', corresponding immunoblot. (b) Two-dimensional gel electrophoresis (Coomassie blue staining) of esophageal cytokeratins, and autoradiograph of corresponding immunoblot (b'). Note that in both epithelia positive reaction is seen for cytokeratins Nos. 5 and 6, whereas basic component No. 4 (b and b') and the small and acidic cytokeratins Nos. 13–16 do not react with antibody K_G 8.13. D, desmin; V, vimentin; IT, intermediate filament-associated component not further characterized. (c) Two-dimensional gel electrophoresis of intestinal cytokeratins (Coomassie blue); (c') autoradiograph of corresponding immunoblot. Note reaction of antibody K_G 8.13 with intestinal cytokeratins Nos. 8 and 18. D, desmin; V, vimentin; IT, intermediate filament-associated component not further characterized.

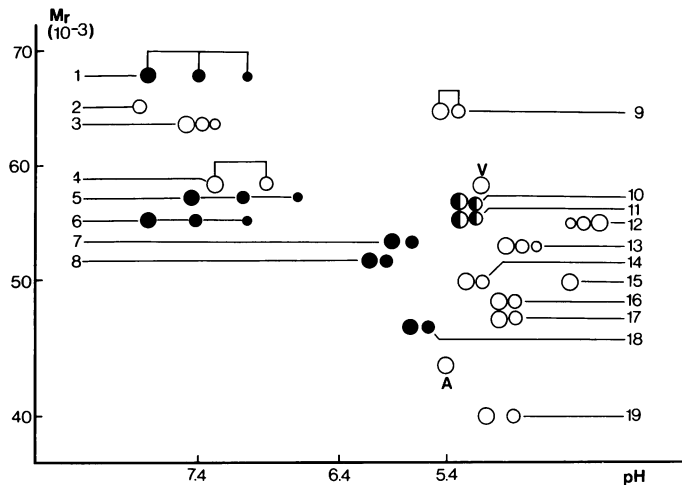


Fig. 4. Schematic diagram showing human cytokeratin polypeptides described so far (Nos. 1–19; M_r , apparent mol. wt.; cf. Schiller *et al.*, 1982) and their reaction with antibody K_G 8.13 in immunoblot experiments: Filled circles, cytokeratins strongly reacting; half-filled circles, weakly reacting components; open circles, non-reacting components. A, α -actin; V, vimentin.

summarized in Figure 4.

A similar pattern of reactivity of antibody K_G 8.13 was found in other higher mammals, as shown for bovine tissues and cell lines in Figure 5a. Of the typical prekeratins of bovine muzzle epidermis, not only the basic components Ia–c, III, IV, and a minor component of apparent mol. wt. 65 000 (Nos. 1–7 as designated by Schiller *et al.*, 1982), but also the acidic cytokeratin VI (No. 13) contained the determinant recognized by the antibody (Figure 5a). As with human cytokeratins, bovine cytokeratins A and D (Nos. 8 and 21) of liver and intestine and some cultured cell lines (BMGE–H and MDBK) were positive. In stratified, non-keratinized epithelia such as tongue and esophagus as well as in certain cell cultures (e.g., line BMGE+H) the basic cytokeratins of mol. wt. 58 000 (No. 7) and 59 000 (No. 6; Franke *et al.*, 1981c; Schiller *et al.*, 1982; Schmid *et al.*, 1982) showed the strongest reaction (Figure 5a).

Reaction with cytokeratin from rodents was restricted to certain basic cytokeratins such as cytokeratins Nos. 9 and 10 of mouse epidermis and cultured keratinocyte-derived cells (Figure 5b, slot 1; cf., Franke *et al.*, 1979d, 1981c). Cytokeratins A and D did not show significant reaction in both rodent species (Figure 5b, slot 2). By contrast, the equivalent of cytokeratin A in a marsupial cell line, PtK₂, did bind antibody K_G 8.13 (Figure 5b, slot 3) as did the corresponding cytokeratin polypeptide of chicken (Figure 5b, slot 4). Antibody binding was also observed with certain amphibian cytokeratins such as two of the major epidermal prekeratin polypeptides of *Xenopus laevis*, a neutral-to-basic component of mol. wt. 59 000 (upper band in Figure 5b, slot 5) which in peptide map analysis appeared to be related to the 'basic sub-family' of polypeptides of mammals (Franz, 1982), and a smaller polypeptide isoelectric with α -actin but slightly larger than it (mol. wt. 44 000).

Immunofluorescence microscopy using antibody K_G 8.13 on a broad variety of tissues of man and cow (Figure 6) showed intense fibrillar staining in all true epithelia examined, including glandular epithelia such as hepatocytes and secretory

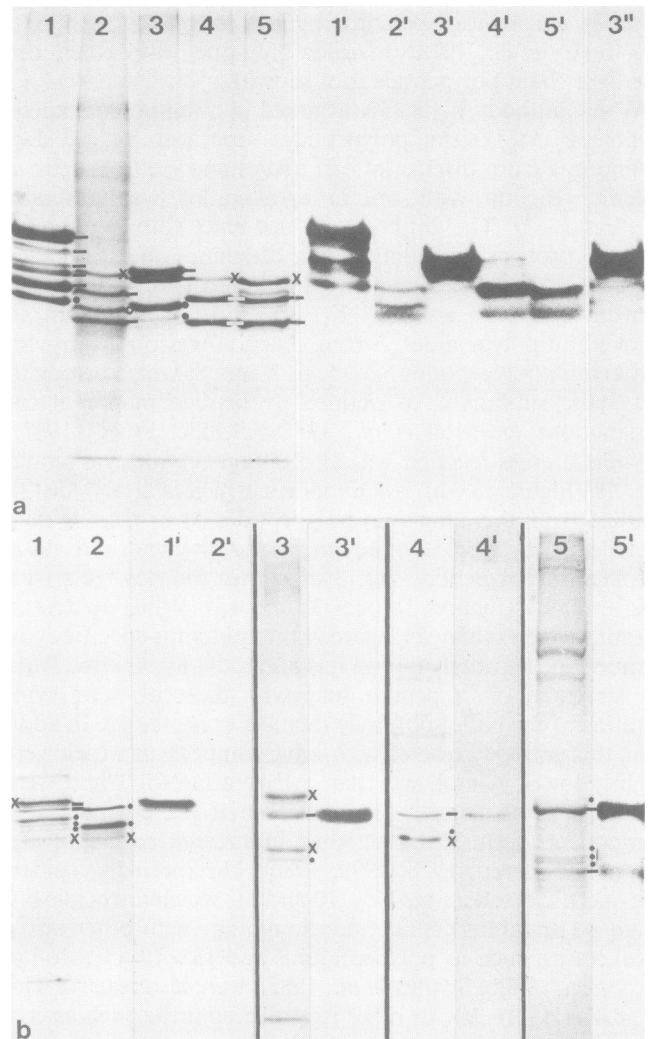


Fig. 5. Immunoblots showing reaction of antibody K_G 8.13 on cytoskeletal polypeptides of cells from diverse vertebrate species. **(a)** Coomassie blue staining of SDS-PAGE of bovine cytoskeletal proteins (slots 1–5) and autoradiography of corresponding immunoblot (slots 1'–5' and 3''). **Slot 1**, bovine muzzle epidermis (prekeratins I, 65.5 K, III, IV, VI, VII); **slot 2**, bovine liver (upper bar, cytokeratin A; lower bar, cytokeratin D; circle, degradation product of cytokeratin A; x, vimentin); **slot 3**, BMGE+H cells (cytokeratins III, IV, VII, and 45.5 K); **slot 4**, BMGE–H cells (cytokeratins A, D; x, vimentin); **slot 5**, MDBK cells (cytokeratins A, D; x, vimentin). Note positive reaction with prekeratins I, 65.5 K, III, IV, VI in epidermis (**slot 1'**) and BMGE+H cells (**slot 3'**) and with cytokeratins A and D in liver (**slot 2'**), BMGE–H cells (**slot 4'**) and MDBK cells (**slot 5'**). Note further detection of small amounts of large keratins (components III and IV) in BMGE–H cells (**slot 4'**) and of cytokeratin A in BMGE+H cells (**slot 3'**, long exposure time) which are not detected in the Coomassie blue-stained gel (slots 3 and 4). **(b)** Cytoskeletal polypeptides from other vertebrate species. **Slots 1–5**, Coomassie blue staining after PAGE; **slots 1'–5'**, autoradiographs of corresponding immunoblots. **Slot 1**, murine keratinocyte cell line HEL containing two large (bars) and three small (dots) prekeratins (upper x, vimentin; lower x, actin); **slot 2**, mouse liver presenting cytokeratins A and D (dots; x, actin). Note positive reaction of only the large and basic prekeratins of HEL cells (**slot 1'**) whereas cytokeratins A and D do not react (**slot 2'**). **Slot 3**, PtK₂ cells (bar, cytokeratin 1; dot, cytokeratin 2; upper x, vimentin; lower x, actin). Note positive reaction only with cytokeratin 1 (**slot 3'**). **Slot 4**, chick intestinal cells (bar, positively reacting cytokeratin in **slot 4'**; dot, polypeptide not reacting with the antibody; x, actin). **Slot 5**, epidermal prekeratin of *X. laevis*. Note positive reaction with two *Xenopus* prekeratin polypeptides (**slot 5'**; bars) whereas other cytokeratins (dots) do not react.

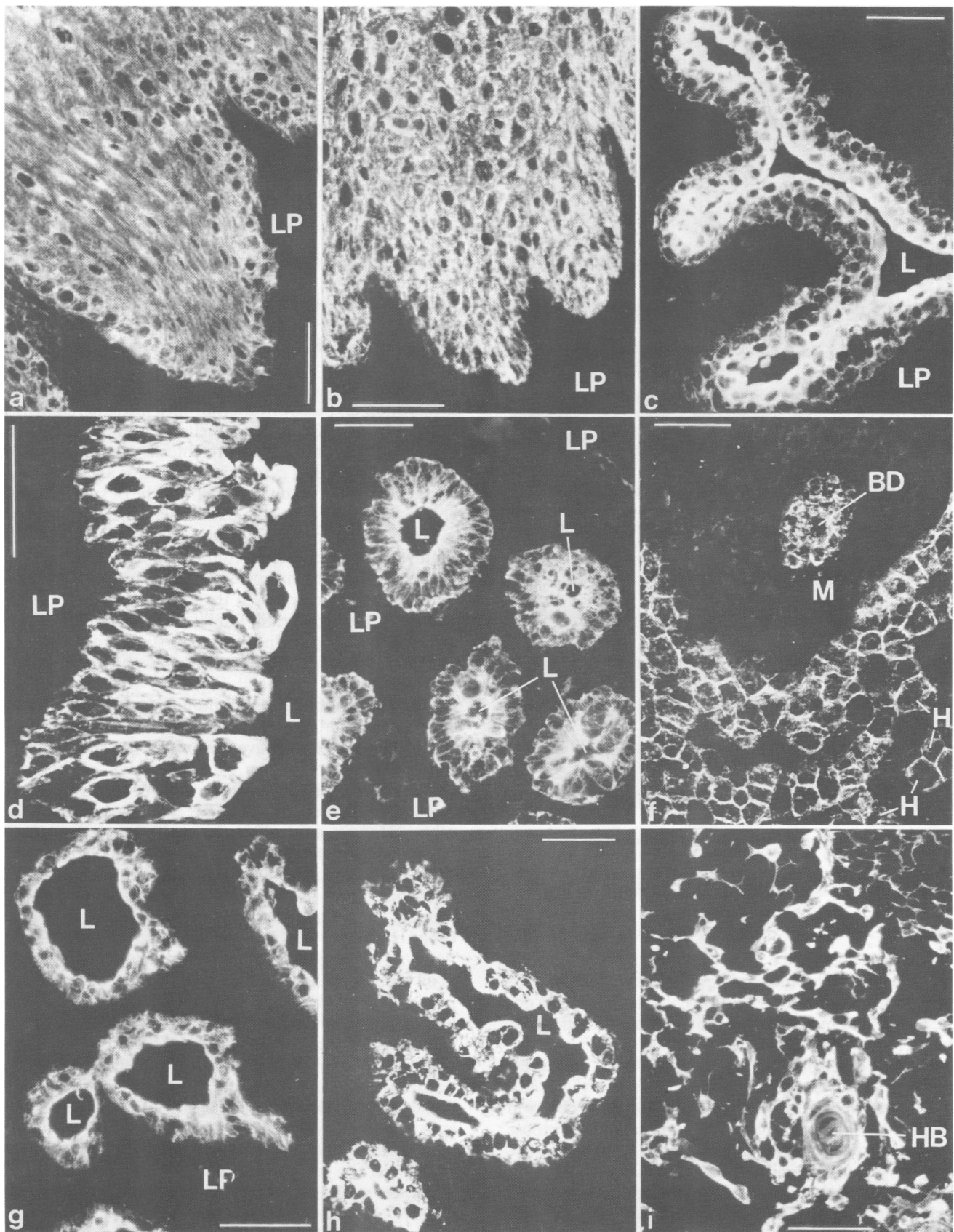


Fig. 6. Immunofluorescence microscopy showing reaction of antibody K_C 8.13 on frozen sections through various human and bovine tissues: (a) bovine muzzle epidermis, (b) bovine tongue, (c) human esophagus, (d) human bladder, (e) human small intestine (crypts), (f) human liver, (g) lactating bovine udder, (h) apocrine glands of human axillary skin, (i) calf thymus. Epithelial cells stained are: epidermis (a), mucosa (b), ducts of esophageal glands (c), urothelium (d), intestinal mucosa (e), bile ducts (BD) and hepatocytes (H) of liver, mammary gland epithelium (g), secretory cells of apocrine glands (h), reticulum epithelium of thymus (i; HB, Hassall bodies). L, lumen. Mesenchymal cells are negative such as *lamina propria* (LP) and perivascular mesenchyme (M) of liver. Bars = 50 μ m.

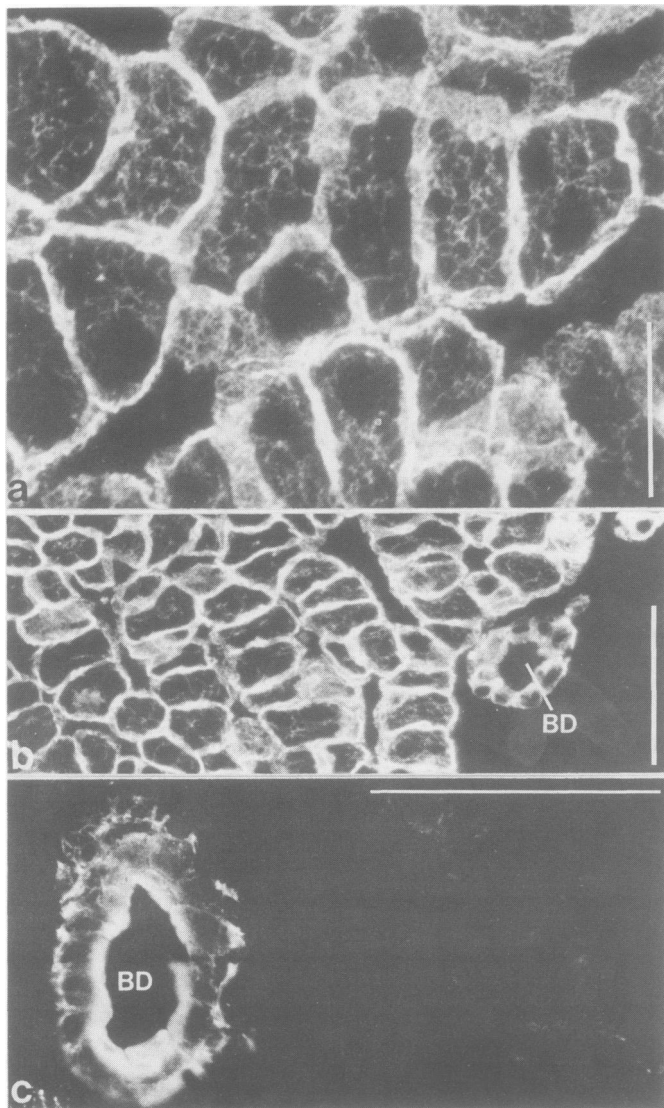


Fig. 7. Immunofluorescence microscopy showing reaction of antibody K_G 8.13 on frozen sections of liver from cow (a,b) and *X. laevis* (c). Note positive reaction in cow (a,b) on hepatocytes and bile ducts (BD) whereas in the amphibian liver (c) only the bile duct epithelia are stained. Bars denote 20 µm (a) and 50 µm (b,c).

cells of lactating mammary gland. In the kidney, collecting ducts as well as distal tubules and large sections of the loop of Henle were positive (not shown). Non-epithelial cells were not stained. On rat and mouse tissues, no significant reaction was seen in certain epithelia such as hepatocytes (not shown). While bile duct epithelial cells were positive in all species examined, the typical cytokeratin fibril meshwork of hepatocytes (Franke *et al.*, 1981a) was decorated by this antibody in man (Figure 6f) and cow (Figure 7a,b) but not in rat, mouse (not shown), and *Xenopus* (Figure 7c).

Similar observations were made on cultured cells. Antibody K_G 8.13 revealed cytokeratin fibril meshworks in all cultured bovine (BMGE + H, BMGE - H, MDBK) and human (HeLa, A-431, MCF-7) cell lines examined (e.g., Figure 8a). Granular mitotic aggregates of cytokeratin material as they are found in certain cell types (Horwitz *et al.*, 1981; Franke *et al.*, 1982b) were also intensely stained with K_G 8.13 (not shown). Most murine cells examined, however, were negative, including rat hepatocytes and hepatoma

MH₁C₁ cells (not shown; for demonstration of cytokeratin fibrils in these cells see Franke *et al.*, 1981d) or showed the phenomenon that only a small proportion of cells of the same culture was stained (compare also Lane and Klymkowsky, 1982). Cytokeratin filaments of PtK₂ cells were usually not decorated in interphase but fibril arrays positively stained with K_G 8.13 were found in mitotic stages (Figure 8b). By contrast, cultured amphibian cells showed well-stained cytokeratin fibrils in all stages of the cell cycle (Figures 8c and 8d).

Discussion

The progress made in recent years in the molecular characterization of intermediate filaments and their differentiation-specific expression in normal tissues and in mouse tumors (Franke *et al.*, 1982b; Holtzer *et al.*, 1982; Osborn *et al.*, 1982) points to the usefulness of monospecific immunochemical reagents defined in molecular terms. Such antibodies with defined specificity may be used for the localization and identification of intermediate filament components in cells and tissues as well as for the determination of molecular relationships of different filament proteins. Consequently, in studies of cytokeratin filaments and epithelial cells two general types of antibodies should be of specific value: (1) antibodies specific for cytokeratin filaments which react with a broad spectrum of members of the cytokeratin protein family and hence all true epithelia and carcinomas; (2) antibodies with a very narrow specificity among the cytokeratin polypeptides which react in the extreme with only one polypeptide of the cytokeratin family and thus only stain cells expressing this antigen. In the present study we have described an immunochemical reagent that fulfills the first requirement, i.e., monoclonal antibody K_G 8.13. Previously described monoclonal antibodies from human patients (Dellagi *et al.*, 1982) or murine hybridomas (Horwitz *et al.*, 1981; Pruss *et al.*, 1981; Lane, 1982) have been either not specific for intermediate filaments of the cytokeratin, i.e., epithelial type (Dellagi *et al.*, 1982; Pruss *et al.*, 1981) or selective in that they react with cytokeratins of some epithelia but not those of others (Horwitz *et al.*, 1981; Lane, 1982).

The detailed characterization of the monoclonal antibody K_G 8.13 by immunoblotting and immunofluorescence microscopy has shown its specificity for proteins of the cytokeratin subclass of intermediate filament proteins as well as for a broad range of different polypeptides of the cytokeratin family. The antibody allows the identification of cytokeratins in all human epithelia so far tested. This is in agreement with previous reports based on other approaches such as immunolocalization with conventional antisera (for references see Introduction), micro-dissection combined with gel electrophoresis and peptide map analysis, and electron microscopic demonstration of typical tonofilaments. Epithelia revealing fibrillar cytokeratin staining include actively secreting cells such as hepatocytes and lactating epithelium of mammary gland, i.e., epithelia in which the cytokeratins have not been recognized by a variety of antibody preparations (for references see Introduction). Antibody K_G 8.13 has also been positive on all carcinomas so far tested (not shown; cf. Moll *et al.*, 1982a, 1982b). Thus, antibody K_G 8.13 presents the broadest range of cross-reactivity between different cytokeratin polypeptides of man so far known and should be of great value in the molecular identification of cytokeratin proteins as well as for the histological and pathological identification of epithelia and carcinomas.

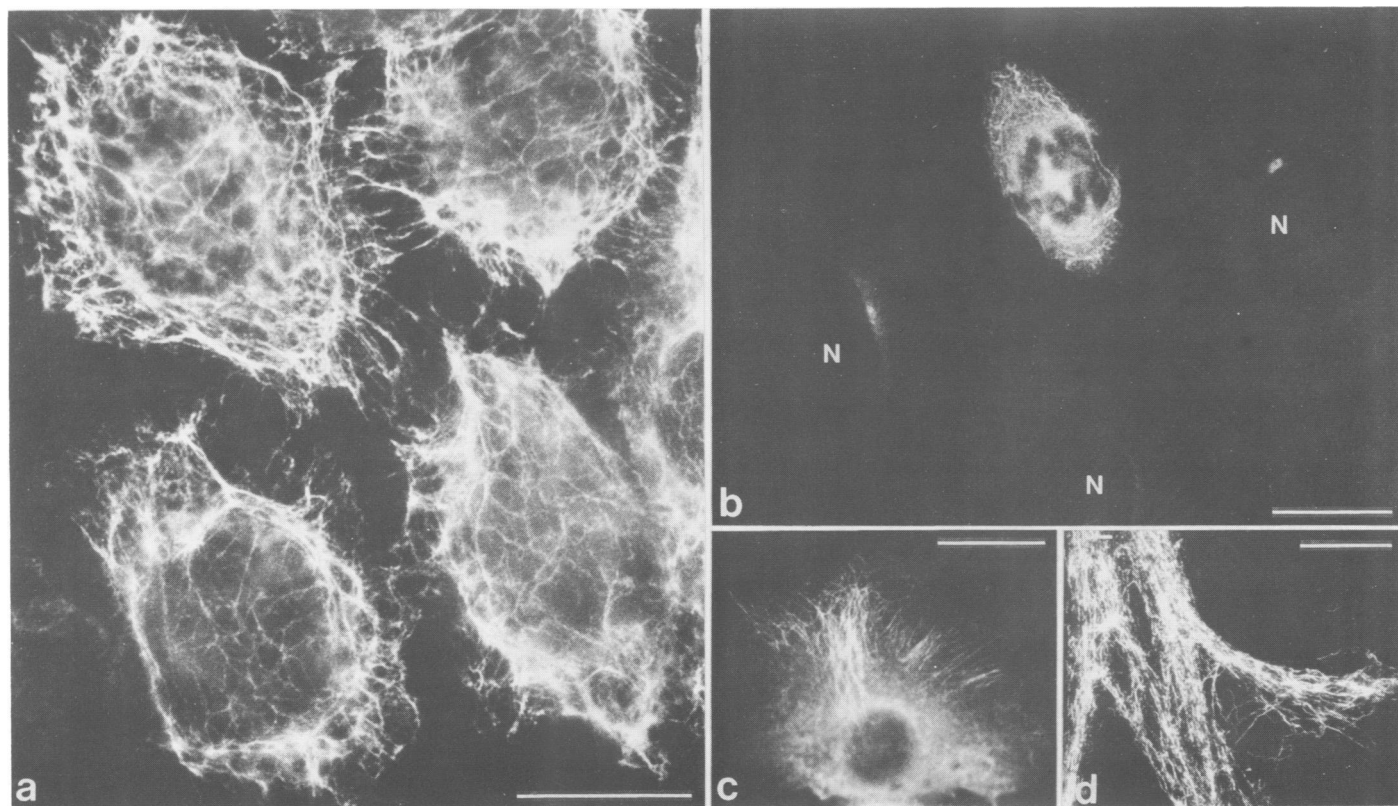


Fig. 8. Immunofluorescence microscopy showing fibrillar staining with antibody K_G 8.13 on cultured human cells (a, HeLa cells), PtK₂ cells (b, from rat kangaroo kidney) which are mostly negative (N designates nuclei of interphase cells) and only in mitotic cells show fibrillar staining (central part of b) and amphibian cells (c, *X. laevis* cell line A6; d, *Triturus cristatus* epidermal cells) which show fibrillar staining throughout the cell cycle. Bars = 20 μ m.

However, antibody K_G 8.13 does not react with all cytokeratin polypeptides. It recognizes a determinant present in several members of the 'basic subfamily' of cytokeratin polypeptides recently defined by peptide map analysis (Schiller *et al.*, 1982; for nucleotide sequence relatedness see also Fuchs *et al.*, 1981). Positive reaction has been found for polypeptides Nos. 1, 5, 6, 7, and 8 of this family whereas no reaction has been found with the neutral-to-basic cytokeratin polypeptides Nos. 3 and No. 4. The only relatively acidic (IEP \leq pH 5.8) cytokeratin polypeptides that have been found to react with K_G 8.13 are cytokeratins Nos. 10 and 11, which are specific for epidermis, and cytokeratin No. 18. These polypeptides have not revealed, in peptide map analyses, relatedness to the basic cytokeratin subfamily (Schiller *et al.*, 1982). This, as well as the cross-reactivity between the acidic prekeratin polypeptides VI of bovine epidermis (Nos. 13a and b; Schiller *et al.*, 1982) and many of the polypeptides of the basic subfamily also indicates the existence of a determinant common to large and basic as well as small and acidic cytokeratins. This may indicate a broad homology between prekeratin polypeptides not detected by peptide map analysis of iodinated proteins and nucleic acid hybridization. Since at least one representative of the group of cytokeratin polypeptides recognized by antibody K_G 8.13 has been found in all of the different human and bovine epithelia studied so far (Schiller *et al.*, 1982; Moll *et al.*, 1982a, 1982b) this antibody may be used as a broad-range cytokeratin-specific reagent for essentially all human cells and tissues of epithelial origin.

While antibody K_G 8.13 has shown good cross-reaction between corresponding cytokeratin polypeptides of man and mammals such as cow (this study), swine, and monkey (not

shown) as well as with phylogenetically distant species such as chicken and amphibia, it reacts to a smaller extent with cytokeratins of rodents (e.g., cytokeratin A corresponding to human component No. 8 is negative in mouse and rat). Currently, we are unable to offer a good explanation for this phenomenon but reduced reactivity against autodeterminants by the species used for production of the hybridoma, i.e. mouse, could be related to this observation.

In view of the growing demand for a cytokeratin-specific, defined antibody in clinical pathology, antibody K_G 8.13 can now be recommended as a 'universal' immuno-histo-diagnostic reagent.

Materials and methods

Preparation of keratin

Prekeratin-enriched preparations, from bovine muzzle, containing the major epidermal polypeptides I–VII (Franke *et al.*, 1978b, 1980; Schiller *et al.*, 1982) were obtained as described (Franke *et al.*, 1981c).

Immunization

Ten (BALB/c x DBA/2)F₁, 3 month-old female mice were injected i.d. and s.c. with total bovine epidermal keratin material (50 μ g in complete Freund's adjuvant) twice with an interval of 2 weeks between injections. Three weeks later, mice received two injections of 50 μ g keratin on two consecutive days. Mouse No. 3 received i.p. and i.v. injections and mouse No. 8 received i.p. injections.

Cell fusion and growth

Three days after the last boost, spleens were removed and 100 x 10⁶ cells from each individual spleen were fused with 20 x 10⁶ NSO/1 myeloma line (Galfre and Milstein, 1981) kindly provided by C. Milstein (MRC, Cambridge, UK) using 41% polyethylene glycol 1500 (Serva, Heidelberg, FRG) as described previously (Eshhar *et al.*, 1979). Following fusion, cells were distributed into six microplates (96 wells each) at a concentration of 5 x 10⁴ viable cells/well.

Hybrid cells selected for growth in the presence of HAT (Littlefield, 1964) were kept in a humidified incubator in the presence of 8% CO₂ in air. The growth medium was Dulbecco's modified Eagle's medium (DMEM high glucose, Gibco) supplemented with 1 mM pyruvate, 2 mM glutamine, penicillin (10 units/ml), streptomycin (20 µg/ml) and 15% heat-inactivated horse serum (HS, Biolab, Jerusalem, Israel). Positive hybrid cultures were weaned out of HAT, cloned and recloned in soft agar and propagated *in vitro* in large volumes of DMEM-HS or *in vivo* as ascites in pristane-treated (BALB/c x DBA/2) mice.

Screening for anti-keratin antibodies by the solid phase radioimmunoassay

The wells of 96 well PVC microtiter plates (Cook Laboratories) were coated with purified prekeratin (50 µl solution of 10 µg/ml in 8 M urea; 5 mM dithioerythritol, DTE) for 2 h at room temperature. After two washes and 1 h incubation in phosphate-buffered saline (PBS)-containing 1% bovine serum albumin (BSA) and 0.05% NaN₃, 50 µl of the hybridoma culture fluid was added to each well and incubated for 2 h at room temperature. After three washes with PBS-containing 1% BSA 50 µl of ¹²⁵I-labelled purified goat anti-mouse Ig (~10⁵ c.p.m.) were added to each well followed by an incubation for 2 h at room temperature. After four washes the amount of bound radioactive antibodies was determined in a gamma counter.

Determination of antibody class

The immunoglobulin class and subclass specificity was determined by the double diffusion test in agar using specific anti-mouse Ig subclass antibodies (Mellory, Springfield, VA).

Tissues and cells

Preparations of tissues from human biopsies and autopsies and from cows, mice, rats, and *X. laevis* have been described (Franke et al., 1981a, 1981b, 1981c; Franz, 1982; Moll et al., 1982a, 1982b; Schiller et al., 1982). Cell cultures were grown as indicated elsewhere (Franke et al., 1979c, 1982; Moll et al., 1982a; Schmid et al., 1982) or as recommended by American Type Culture Collection (Rockville, MD).

Cytoskeletal preparations

Cytoskeletal fractions were prepared from the various cells and tissues as described (Franke et al., 1978b, 1981a, 1981b, 1981c; Renner et al., 1981; Schiller et al., 1982). Human myometrial tissue and cytoskeletal material obtained therefrom (e.g., Jackson et al., 1980) was used for comparison.

Gel electrophoresis and antibody binding

For separations of proteins by SDS-polyacrylamide gel electrophoresis a modification of the system of Laemmli (1970) was used, employing the electrode buffer of Thomas and Kornberg (1975) and an acrylamide: N,N'-methylene bis-acrylamide ratio of 30:0.15. For two-dimensional gel electrophoresis, proteins were either separated according to O'Farrell (1975) or O'Farrell et al. (1977). After separation, polypeptides were transferred electrophoretically onto nitrocellulose paper and characterized by reaction with antibodies (Towbin et al., 1979). SDS-polyacrylamide gels of the separated polypeptides were prewashed for 10 min in the blotting buffer containing 190 mM glycine, 25 mM Tris-HCl pH (8.3) and 20% (v/v) methanol, prior to electrophoretic transfer onto nitrocellulose paper. The transfer was for 2 h using a constant current of 350 x 10⁻³ amps at 4°C. The nitrocellulose paper was saturated with a solution containing 1% BSA in PBS (minimum 2 h). Antibodies in 8–12 ml PBS containing 2% BSA (~0.1 mg IgG/ml) were added to the nitrocellulose paper which was then put into a plastic bag and allowed to react for 2 h at room temperature under gentle rotation. After several 15 min washes, the paper sheets were washed in the following solutions: (1) 0.5% Triton X-100 PBS; (2) 0.637 M NaCl, 2.7 mM KCl, 7 mM Na₂HPO₄, 1.5 mM KH₂PO₄ (pH 7.2; 'high salt buffer'); (3) PBS; (4) PBS containing 0.1% BSA. The immunoglobulins retained on the paper sheets were detected by incubation with ~1 µCi of ¹²⁵I-labelled protein A from *Staphylococcus aureus* (New England Nuclear, Boston, MA) in 150 ml of PBS containing 0.1% BSA for 2 h at room temperature under gentle shaking. The nitrocellulose paper sheets were washed several times for 15–30 min, first in PBS containing 0.1% BSA; then in PBS containing 0.1% Triton X-100 and PBS containing 0.5% Triton X-100, then in 'high salt buffer' (see above), and finally in PBS. After drying at 80°C the paper sheets were exposed to X-ray film.

Immunofluorescence microscopy

Cryostat sections (3–5 µm) through frozen tissues and whole mount preparations of cultured cells grown on cover slips were processed for indirect immunofluorescence microscopy as described (Franke et al., 1979a, 1979b, 1980).

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