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Antigenic Interrelationship Between the 40-Kilodalton Cytokeratin Polypeptide and Desmoplakins

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We describe here antigenic cross-reactivity between the human 40-kilodalton cytokeratin polypeptide [Moll et al] and components of bovine desmosomal plaque, namely desmoplakins I and II. This relationship was revealed by an antibody (KM 4.62), raised against cytoskeletal preparation of cultured human breast adenocarcinoma cells (MCF-7) and selected by immunoblotting and immunofluorescent labeling. In cultured human cells that contain the 40-kD cytokeratin, antibody KM 4.62 labeled arrays of filaments throughout the cytoplasm. This antibody labeled the basal layer of nonkeratinizing squamous epithelia as well as various simple (normal and malignant) epithelia and epithelial elements of the thymus. In liver tissue, labeling was obtained only in bile ducts and canaliculi but not in the hepatocytes.

In bovine cells and tissues, on the other hand, immunofluorescent labeling with antibody KM 4.62 was strictly confined to desmosomes. This was verified by double immunolabeling with both antibody KM 4.62 and specific cytokeratin or desmosomal antibodies. Immunoblotting analysis indicated that the former antibody reacts specifically with the high molecular weight components of the bovine desmosomal plaque, namely desmoplakins I and II. These immunochemical results suggest that bovine desmoplakins share same structural relationship with the human acidic, 40-kD cytokeratin.

Key words: monoclonal antibody, cytokeratins, desmoplakins

INTRODUCTION

Recent studies on the cytoskeleton of cultured cells and tissues have focused much attention on the cytokeratins, a family of epithelia-specific intermediate filament polypeptides [Lazarides, 1980, 1982; Anderton, 1981]. Biochemical studies have indicated that there are about 20 different cytokeratin polypeptides expressed in mammalian epithelial tissues [Franke et al, 1981a; Moll et al, 1982a; Quinlan et al, 1985], which may be subdivided into two distinct subfamilies: the acidic (type I) and the basic (type II) polypeptides [Fuchs and Green, 1978; Schiller et al, 1982; Kim et al, 1983; Hanukoglu and Fuchs, 1983]. Examination of a large variety of epithelia indicated that the expression of cytokeratins is cell-type restricted and that in each cell only a few polypeptides (2–10) are present [Franke et al, 1981a; Moll et al, 1982a; Quinlan et al, 1985]. Moreover, it was shown that the combination of acidic and basic polypeptides is essential for the assembly into intermediate filaments [Moll et al, 1982a,b; Schiller et al, 1982]. Recently, batteries of antibodies (mostly monoclonal) specific for one or only a few cytokeratins have been prepared in several labora-

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tories. This enabled the localization of specific polypeptides in cells and tissues at high levels of sensitivity, specificity, and resolution using various immunohistochemical methodologies [Franke et al, 1979a,b; Gigi et al, 1982; Tseng et al, 1982; Debus et al, 1982; Osborn, 1983; Cooper et al, 1984].

Examination of intermediate filaments in epithelial cells using either electron microscopy or immunocytochemical labeling revealed that the elaborate mesh-works of filament bundles that are abundantly distrubuted throughout the cytoplasm are often attached to the plasma membrane at desmosomal or hemidesmosomal junctions [Skerrow and Skerrow, 1980; Moll and Franke, 1982; Geiger et al, 1983]. Previous electron microscopic examinations demonstrated that cytokeratin-containing tonofilaments associate with electron-dense plaques at the cytoplasmic faces of the desmosomal membranes, forming typical mirror image configurations [Farquhar and Palade, 1963; Kelly, 1965; Kelly and Shienvold, 1976; Staehelin, 1974]. Recent studies with epidermal desmosomes purified mostly according to the approach of Skerrow and Matoltsy [1974], have revealed a group of specific desmosomal proteins whose general biochemical properties and gross localization were determined. Among those are specific constituents of the desmosomal plaque (desmoplakins I, II, and III with approximate MW of 250 kD, 215 kD, and 80 kD) [Franke et al, 1981b, 1983; Mueller and Franke, 1983; Gorbsky et al, 1985; Cowin et al, 1985] as well as several membrane glycoproteins specific for this type of cell junction [Gorbsky and Steinberg, 1981; Cohen et al, 1983; Cowin and Garrod, 1983; Cowin et al, 1984]. Both desmosomal plaque proteins (desmoplakins) and desmosomal membrane proteins (desmogleins or desmocollins) were molecularly distinguishable from the various cytokeratin polypeptide both by biochemical and immunochemical criteria. It has nevertheless been recently shown that some similarity in amino acid composition exists between keratins and desmoplakins [Jorcano et al, 1984; Kapprell et al, 1985], though the significance of this observation could not be definitively evaluated owing to the absence of sequence data or detailed immunochemical results.

In the present study, we report that a monoclonal antibody specific for only one human cytokeratin polypeptide (the acidic 40-kilodalton polypeptide No. 19) reveals an epitope shared by this cytokeratin and bovine desmoplakins I and II. Moveover, while positive immunofluorescent staining of human tissues was restricted only to those cells that contain the 40-kD polypeptide, all bovine epithelia examined were positively labeled with this antibody in accordance with the ubiquitous occurrence of at least desmoplakin I in all epithelia. The significance of this structural interrelationship and its possible involvement in cytokeratin-desmoplakin interactions will be discussed.

MATERIALS AND METHODS

Cells and Tissues

Cultured cell lines used in this study included (1) human breast adenocarcinoma (MCF-7); (2) human colon adenocarcinoma (HCT); (3) human lung carcinoma (OAT); (4) human epidermoid carcinoma of the vulva (A-431); (5) Human cervical adenocarcinoma (HeLa) [for references see Moll et al, 1982a; Quinlan et al, 1985]; (6) bovine mammary gland epithelium (BMGE) [Schmid et al, 1983]; (7) Bovine Madin-Darby kidney epithelium, (MDBK) [Madin and Darby, 1958]. All cells were maintained in culture in Dulbecco's Modified Eagles Medium (DMEM), supplemented with 10% fetal calf serum and antibiotics and maintained at 37°C under humid atmosphere of 7% CO_2 in air. For immunofluorescent labeling, cells were routinely cultured on square (18 mm) or round (12 mm) glass coverslips.

Human tissues from surgical specimens were rapidly frozen in liquid nitrogen-cooled isopentan, and stored at −80°C. Bovine tissues were dissected within 15-30 minutes after slaughter, frozen as above and stored at −80°C until use [Altmannsberger et al, 1981; Franke et al, 1979a].

Cytoskeletal and Desmosomal Preparations

Isolation of cytoskeletal proteins from cultured MCF-7 cells was carried out by the detergent/high-salt extraction as described [Franke et al, 1978]. The predominant components in this preparation were cytokeratins Nos. 8, 18, and 19 (MW, 52.5 kD, 45 kD, and 40 kD, respectively) [Moll et al, 1982a; Quinlan et al, 1985] with only limited contamination by actin. Desmosomes were isolated from bovine snout according to the modification of the Skerrow and Matoltsy method [1974] described by Mueller and Franke [1983] and desmoplakins kindly provided by P. Cowin and W. Franke, the German Cancer Research Center, Heidelberg, FRG.

Monoclonal Antibodies

Cytoskeletal proteins of MCF-7 cells containing 50 μg protein were suspended in PBS, emulsified in Complete Freund's Adjuvant, and injected into the foot pads of Balb/C mice. The animals were boosted after 2 weeks, and 3 weeks later they received two intraperitoneal injections of 50 μg protein on 2 consecutive days [Gigi et al, 1982]. Three days following the last injection, the spleens were removed, and the splenocytes fused with nonproducing mouse myeloma cells (NSO) as described [Gigi et al, 1982; Eshhar et al, 1980]. Screening for antibody-producing cultures was carried out by indirect immunofluorescent labeling of cultured MCF-7 cells. Positively reacting hybridoma cells were cloned in agar and positive clones reselected by immunofluorescent labeling. Antibody solutions for the various assays consisted of either

culture supernatants or ascites fluids. Another monoclonal antibody used here was the broadly cross-reacting cytokeratin antibody K_G 8.13 [for details see Gigi et al, 1982].

Immunochemistry and Immunocytochemistry

Cells and tissues were immunofluorescently labeled by the indirect immunofluorescent method using rhodamine- or DTAF-labeled goat anti-mouse Fab antibodies [Brandtzaeg, 1973; Geiger and Singer, 1979]. A standard fixation of cultured cell monolayers included 5 minutes in methanol at −20°C followed by 1 minute in cold acetone. Frozen tissue blocks were sectioned (4–5 μm) at approximately −20°C with a Frigocut 2700 cryostat (Jung-Reichert, FRG). The sections were fixed in cold acetone and then dried and immunolabeled [Franke et al, 1979c]. Double immunofluorescence was carried out using, in conjunction, the murine monoclonal antibodies and rabbit antibodies to either bovine epidermal cytokeratins or to bovine epidermal desmoplakins. The latter antibody was kindly provided by W. Franke and coworkers at the German Cancer Research Center, Heidelberg, FRG.

For immunoblotting analysis, cytoskeletal proteins were separated by polyacrylamide gel electrophoresis [Laemmli, 1970] and electrophoretically transferred onto nitrocellulose sheets [Towbin et al, 1979]. Positively reacting bands were identified by indirect radioimmunolabeling using the primary antibodies KM 4.62 or monoclonal antibody to desmoplakins 1 and 2,2.15 [Cowin et al, 1985] followed by ^{125}I-labeled goat antibodies to mouse immunoglobulins. (The monoclonal antibody DP1 and 2,2.15 was kindly provided by W. Franke).

RESULTS

Polypeptide Specificity of Monoclonal Antibody KM 4.62

The specific reactivity of antibody KM 4.62 was examined by immunoblotting analysis using different cells and tissues (Fig. 1). The sources of cytoskeletal proteins examined included human foot sole epidermis (contains polypeptides Nos. 1, 2, 5, 6, 9, 10, 11, 14, and 16), cultured A-431 cells (contain polypeptides Nos. 5, 8, 13, 15, 17, and 18 as well as small amounts of Nos. 6, 7, 14, and 16) and MCF-7 cells (contain polypeptides Nos. 8, 18, 19). Together, most cytokeratin polypeptides are represented with the exception of the cornea-specific polypeptides Nos. 3 and 12 as well as No. 4. Examination of the immunoblots indicated that antibody (KM 4.62) reacted only with the 40-kD polypeptide No. 19 and even after long exposure of the autoradiograms no additional reactivity was detected.

Immunofluorescent Labeling of Human Cells and Tissues With Antibody KM 4.62

Immunofluorescent labeling of MCF-7 cells with antibody KM 4.62 revealed elaborate networks of filaments and filament bundles distributed throughout the cytoplasm (Fig. 2A). These filaments often reached peripheral sites of apparent cell-cell contact, although the desmosomal junctions themselves appeared unlabeled. Double labeling of the same cells with both KM 4.62 and with rabbit anti-keratin antibodies (Fig. 2B) yielded overlapping staining patterns. We have further labeled other cultured epithelial cells from carcinomas of the colon (Fig. 2C) and lung (Fig. 2D). In all cells examined, an elaborate filamentous cytoplasmic staining was apparent. In contrast, cultured human epidermoid carcinoma of the A-431 cell line was negative (Fig. 2E) in accord with the immunoblotting results shown above (Fig. 1, slot No. 2). On the basis of these observations, we have concluded that antibody KM 4.62 is a reliable immunocytochemical probe for the human 40-kD cytokeratin and that the latter is uniformly distributed throughout the keratin network of all cells studied.

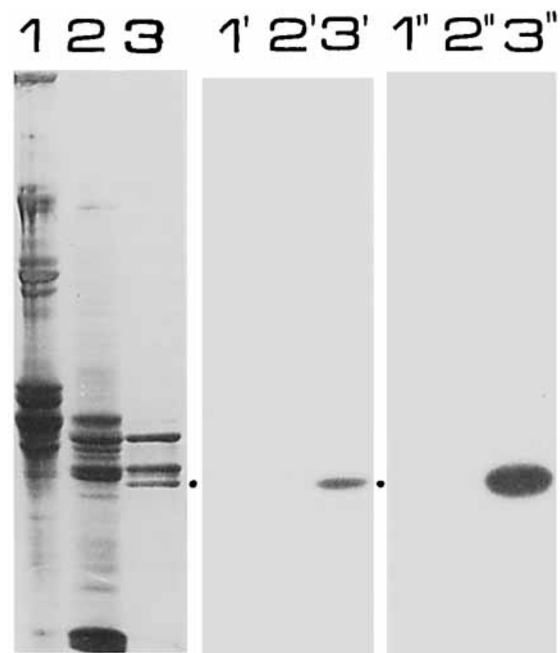

Fig. 1. Immunoblotting analysis of antibody KM 4.62 with cytoskeletal fraction of human tissue including foot-sole epidermis (slot 1); cultured vulva carcinoma A-431 cells (slot 2); cultured breast adenocarcinoma MCF-7 cells (slot 3); (1–3) Coomassie blue staining of the polyacrylamide electrophoretic gel; (1′–3′) autoradiogram of the immunoblot, exposed for 6 hours; (1″–3″) autoradiogram of the same immunoblot, exposed for 48 hours. Notice the exclusive reactivity of antibody KM 4.62 with the 40-kD polypeptide (No. 19, marked by the asterisks).

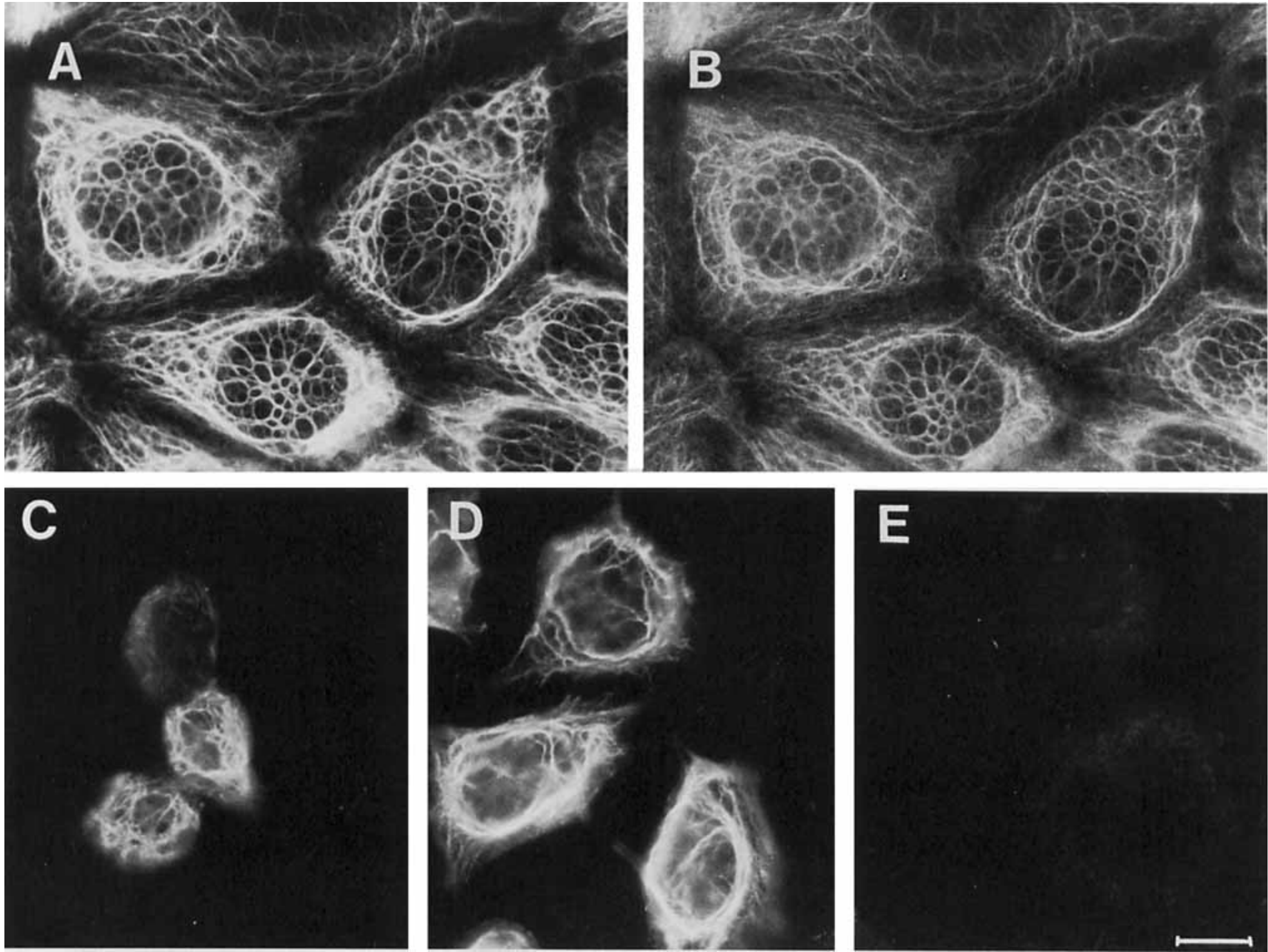

Fig. 2. Immunofluorescent labeling of cultured human cells with antibody KM 4.62. A,B: Double immunofluorescent labeling of the same MCF-7 breast adenocarcinoma cells with antibody KM 4.62 and polyclonal rabbit antibody against epidermal keratins, respectively. C–E: Immunofluorescent labeling with antibody KM 4.62 of cultured human cell lines including colon carcinoma HCT cells. C, human lung carcinoma OAT line; D, human epidermoid carcinoma of the vulva; E, A-431. Bar = 10 μm.

Localization of the 40-kD Cytokeratin Polypeptide in Human Tissues

Immunofluorescent labeling of stratified, nonkeratinizing human epithelia (esophagus and exocervix) with antibody KM 4.62 revealed strong reactivity with the basal layer only (Fig. 3A and C). In contrast, a broadly cross-reacting cytokeratin antibody (K_G 8.13) stained all layers in both tissues (Fig. 3B and D, respectively). Keratinizing epithelia such as the epidermis were largely negative showing strong immunofluorescent labeling only of epidermal appendages such as sweat glands and their ducts (Fig. 3E). Thymic epithelial cells showed extensive labeling, including the cornifying foci of the Hassall bodies (Fig. 3F).

Immunohistochemical examination of human breast, colon, and endocervix tissues showed extensive labeling of the epithelial elements (Fig. 4A–D). In liver, hepatocytes were negative, whereas bile ducts were brightly stained (Fig. 4E). As a control, labeling of liver tissue with antibody K_G 8.13 was positive on both hepatocytes and bile ducts (Fig. 4F). The reactivity of antibody KM 4.62 and the broad spectrum antibody K_G 8.13 on a large variety of human tissues is summarized in Table I.

Monoclonal Antibody KM 4.62 Reacts With Desmoplakins in Bovine Cells and Tissues

Immunofluorescent labeling of frozen sections of bovine snout (Fig. 5A,B), colon (Fig. 5C), and liver (Fig. 5D) by antibody KM 4.62 revealed an extensive punctuate peripheral staining pattern, clearly discernable from the typical keratin staining of the same tissues. This staining was very similar to the one obtained in these tissues with desmoplakin-specific antibodies. To study the spatial relationships between the structures stained in bovine cells with KM 4.62 and desmosomes at a higher

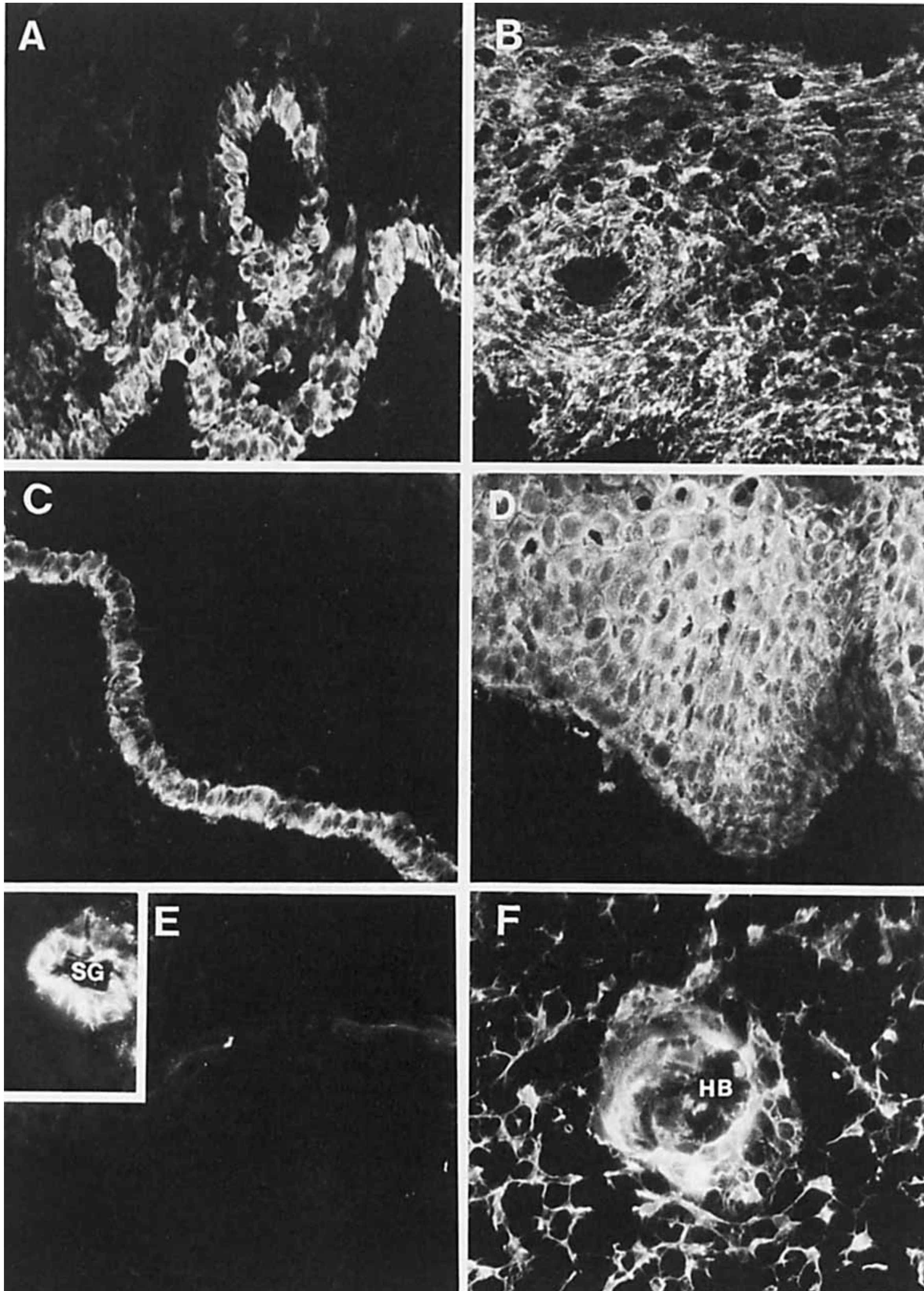

Fig. 3. Immunofluorescent labeling of frozen sections of different human tissues with monoclonal antibodies KM 4.62 and K_G 8.13. A,B: Labeling of human esophagus with antibodies KM 4.62 and K_G 8.13, respectively. C,D. Labeling of human exocervix with the respective two antibodies. E: Labeling of skin epidermis with antibody KM 4.62 (the insert shows positively reacting sweat gland duct [SG]). F. Labeling with antibody KM 4.62 of human thymus showing positive reaction on the reticular epithelial cells and the Hassall bodies (HB). Notice particularly the labeling of the basal layer(s) in the esophagus and exocervix and the absence of labeling from the keratinizing epithelium of the epidermis. Bar = 25 μm.

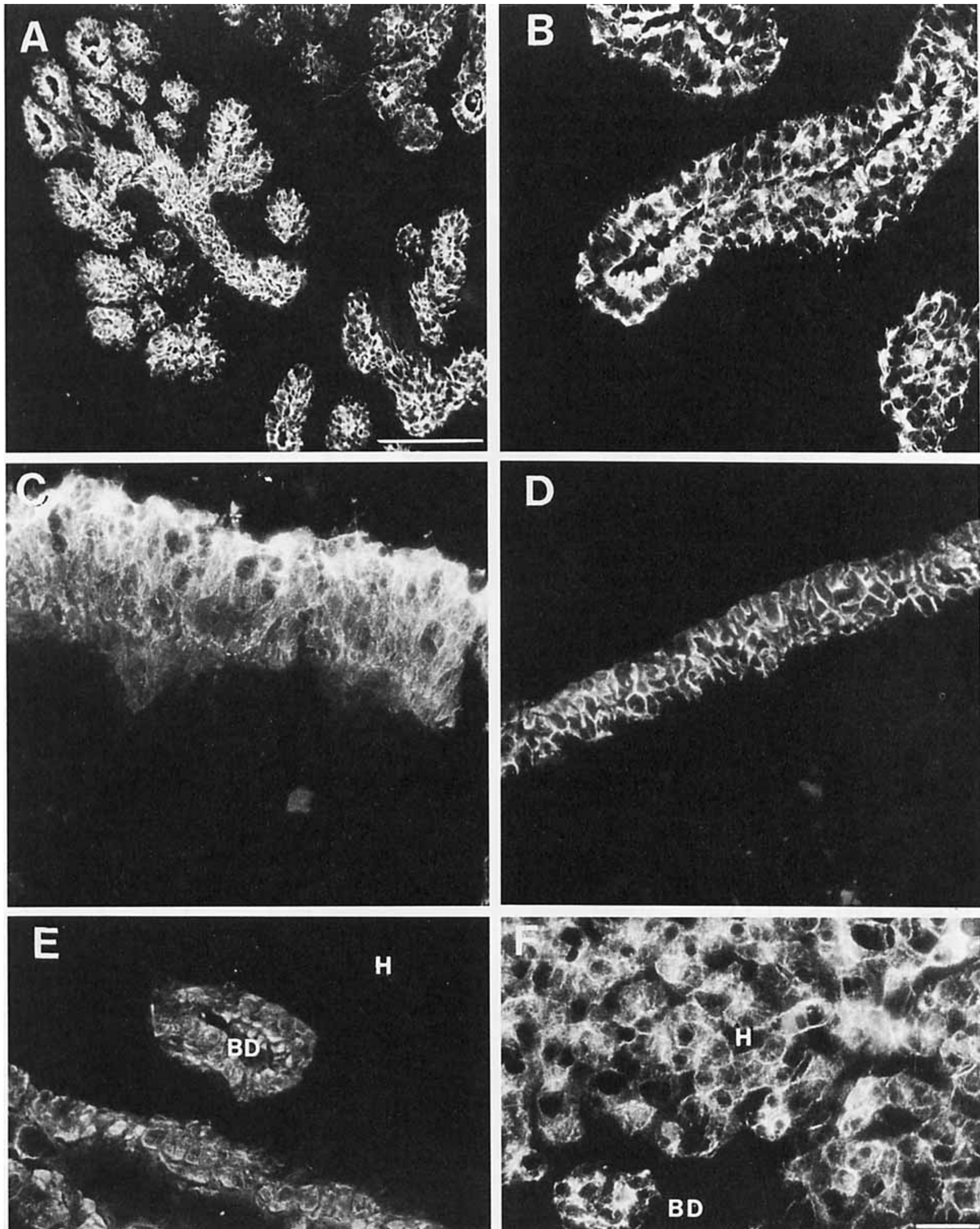

Fig. 4. Immunofluorescent labeling of human simple epithelia with monoclonal antibodies KM 4.62 (A–E) or K_G 8.153 (F). The tissues labeled included breast (A,B), colon (C), endocervix (D), liver (E,F). Notice the extensive labeling of the various simple epithelia and the apparent absence of labeling from the hepatocytes (hepatocytes contain only cytokeratins Nos. 8 and 18 and are positively labeled only with the broadly cross-reacting antibody K_G 8.13) (F). H, hepatocytes; BD, bile ducts. Bar = 25 μm.

TABLE I. Immunohistochemical Staining With Anti-Cytokeratin mAbs*

Tissue	K_G 8.13	KM 4.62	Presence of polypeptide No. 19[a]
Skin			
Epidermis	+	−	−
Sweat gland	+	+	+
Gastrointestinal tract			
Salivary gland	+	+	+
Esophagus: basal layer	+	+	(+)
Esophagus: suprabasal layers	+	−	
Large intestine, mucosa	+	+	+
Liver: hepatocytes	+	−	−
Liver: bile ducts	+	+	+
Colon	+	+	+
Female genital tract			
Exocervix: basal layer	+	+	(+)
Exocervix: suprabasal layer	+	−	
Endocervix	+	+	+
Mammary gland duct	+	+	+
Thymus (incl. Hassal bodies)	+	+	+
Human cell lines			
MCF-7 (adeno carcinoma of breast)	+	+	+
HCT (colon carcinoma)	+	+	+
OAT (lung cells carcinoma)	+	+	+
A-431 (epidermoid carcinoma of vulva)	+	−	−
HeLa (cervical adenocarcinoma)	+	−	−

*+, Component present in minor or variable amounts.
[a]Moll et al [1982a], Quinlan et al [1985].

level of resolution, we have double-labeled different cultured bovine cells (BMGE and MDBK) with antibody KM 4.62 (Fig. 6A,C,E) and either cytokeratin (Fig. 6B) or desmoplakin (Fig. 6D,F). Examination of the paired patterns indicated that the bright spots at the cell periphery stained with KM 4.62 coincided with the termini of cytokeratin bundles (Fig. 6A,B) as previously shown for desmoplakin-cytokeratin pairs in the same cell type [Geiger et al, 1983] or in cultured keratinocytes [Jones and Goldman, 1985]. Double labeling with KM 4.62 and with antibodies pointed to an essentially complete coincidence (Fig. 6C–F).

To identify further the cross-reacting molecules in bovine tissues, we have run an SDS polyacrylamide gel electrophoresis of purified desmosomal preparation (Fig. 7) and examined the reactivity of the various bands by immunoblotting analysis. As shown in Figure 7, antibody KM 4.62 reacted with two high molecular weight bands coinciding with desmoplakins I and II. This observation indicates that the two desmoplakins are antigenically cross-reactive and that one of the common epitopes shared by the two is also present on the human 40-kilodalton cytokeratin.

DISCUSSION

One of the unique and intriguing features of intermediate filaments is the apparent contrast between their remarkable molecular diversity on the one hand and their close structural and biophysical similarity on the other [Lazarides, 1980; Steinert et al, 1983; Gown and Vogel, 1982; Hanukoglu and Fuchs, 1982; Pruss et al, 1981]. The diversity of intermediate filaments is expressed at different levels: As established in numerous studies, there are five biochemically, antigenically, and developmentally distinct families of intermediate filament subunits [Lazarides, 1980, 1982; Anderton, 1981; Osborn and Weber, 1982]. Further complexity is detected in the cytokeratin family, which is present in epithelial cells. Detailed biochemical analysis indicated that different epithelia contain nearly 20 distinct polypeptides ranging in molecular weight from 40 to 69 kD [Schiller et al, 1982; Moll et al, 1982a]. Further examination of the cytokeratin family by biochemical and molecular-genetic approaches indicated that the battery of cytokeratin polypeptides may be subdivided into two major subfamilies, one consisting of relatively large and basic polypeptides and the other of small and acidic ones [Schiller et al, 1982; Kim et al, 1983; Hanukoglu and Fuchs, 1983]. Immunochemical studies employing mostly monoclonal antibodies supported these results and revealed epitopes shared by all or several polypeptides of each cytokeratin subfamily [i.e., Gigi et al, 1982] as well as antigenic determinants that are present on only one or few cytokeratin polypeptides [Tseng et al, 1982; Cooper et al, 1984; Wu and Rheinwald, 1981; Bartek et al, 1985]. Among

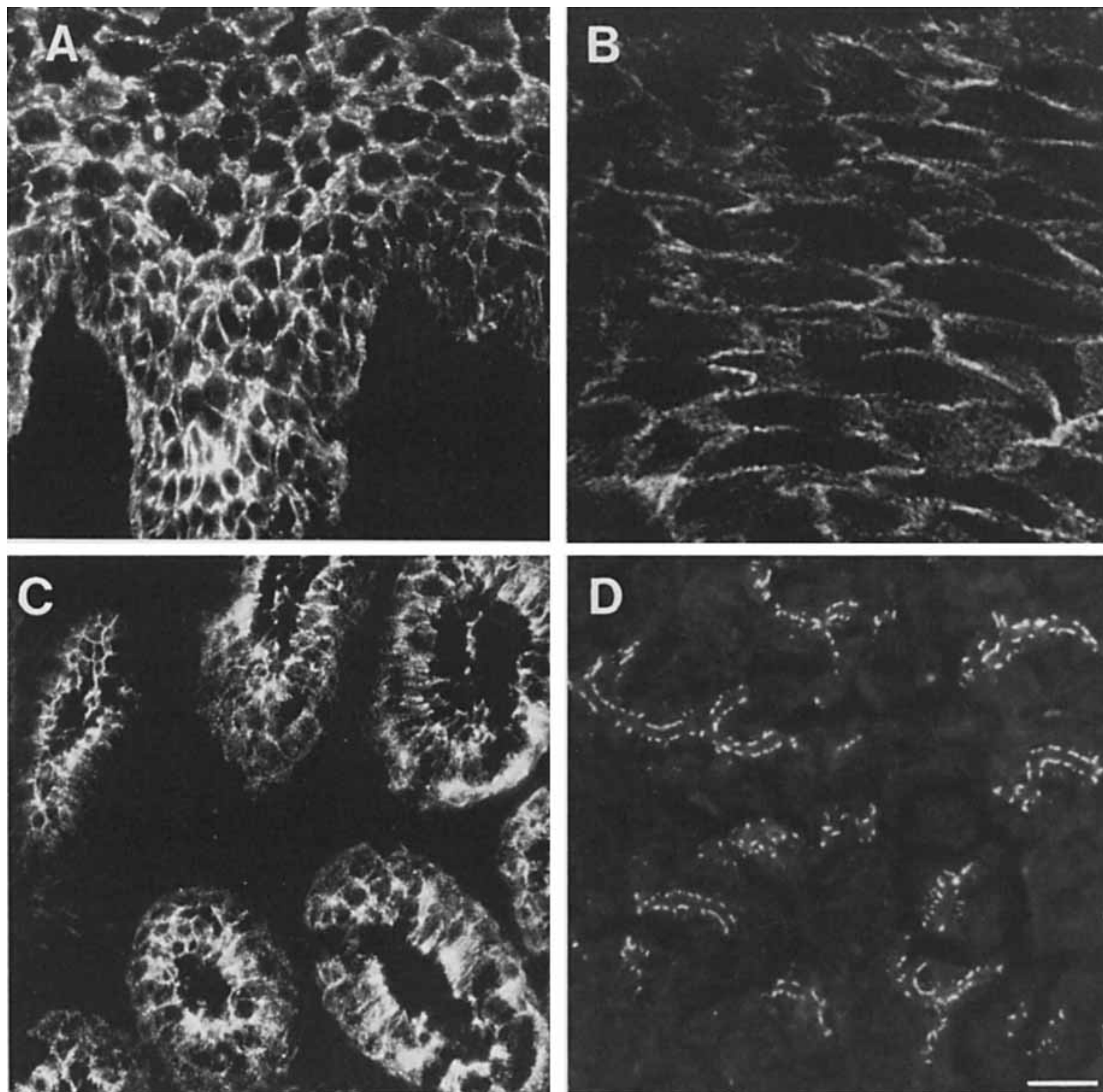

Fig. 5. Immunofluorescent labeling of various bovine epithelia with antibody KM 4.62: snout (A and B different magnifications), small intestine (C), liver (D). Notice that the labeling pattern in bovine tissues is primarily punctuated and confined to the cell periphery. Bar = 25 μm.

these is a recently described monoclonal antibody that reacts with the 40-kD polypeptide [Bartek et al, 1985]. The results largely agree with ours, though the antigenic relationship to desmoplakins is not reported. It should be pointed out that in addition to the intrinsic constituting polypeptides of intermediate filaments, these fibers were reported to be associated with different proteins (intermediate filament associated proteins), which may be involved in their bundling or their binding to other cellular structures such as the plasma membrane.

In the present study, we have used a unique monoclonal antibody that reacts with an epitope present on only one cytokeratin polypeptide in human tissues but recognizes a nonkeratin, yet keratin-related, molecule in all bovine epithelia. We would like to discuss here separately two issues illuminated by our observations, namely the specific distribution of the 40-kD polypeptide in human tissues and cells and the apparent antigenic cross-reactivity observed between this polypeptide and bovine desmoplakins.

Staining of a large variety of normal and malignant human tissues, part of which was presented here, indicated that antibody KM 4.62 is a most useful reagent for the immunohistochemical localization of the human 40-kD cytokeratin. The information obtained from such labeling experiments corroborated the biochemical data already available on the cytokeratin composition on different epithelia [Moll et al, 1982a; Quinlan et al, 1985;

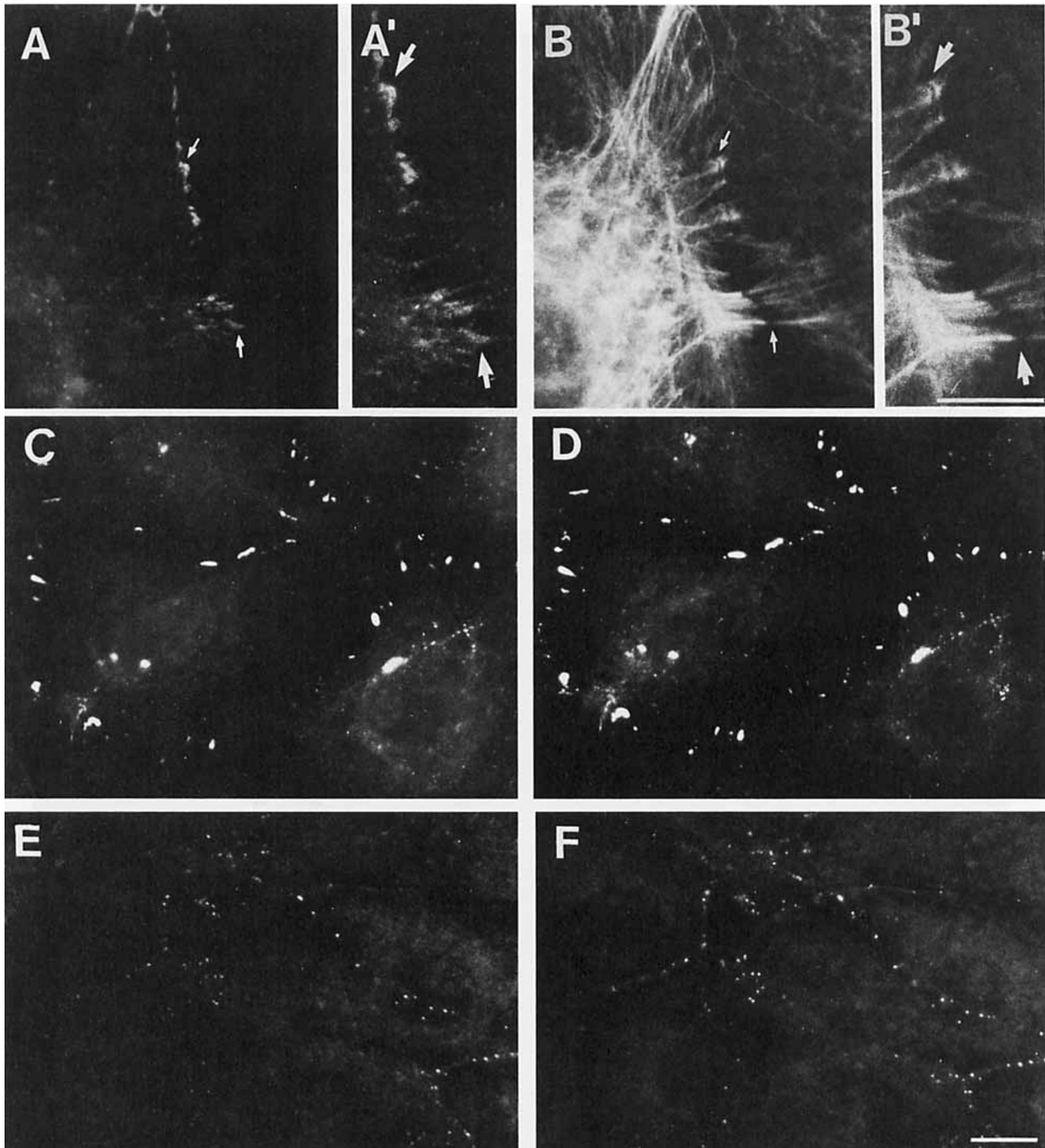

Fig. 6. Double immunofluorescent labeling of culture bovine cells including BMGE (A–D) and MDBK (E,F) with antibody KM 4.62 (A,A′,C,E) and with either rabbit anti-cytokeratin (B,B′) or rabbit anti-desmoplakin (D,F), respectively. Notice that the spots labeled by antibody KM 4.62 coincide with the termini of cytokeratin arrays often corresponding to the apparent junctional gap in the cytokeratin labeling (see matching arrows in A and B and the enlarged regions in A′ and B′). The spots labeled with antibody 13 match precisely the desmoplakin positive structures. Bars = 10 μm.

see also Table I]. However, it also revealed further details that could not be resolved by gel electrophoresis. For example, the immunofluorescent results showed that the basal cell layer only in nonkeratinizing squamous epithelia was positive, while the suprabasal layers were not. Another example which became apparent in a recent series of studies on the cytokeratins of salivary glands included the positive labeling of ductal elements with

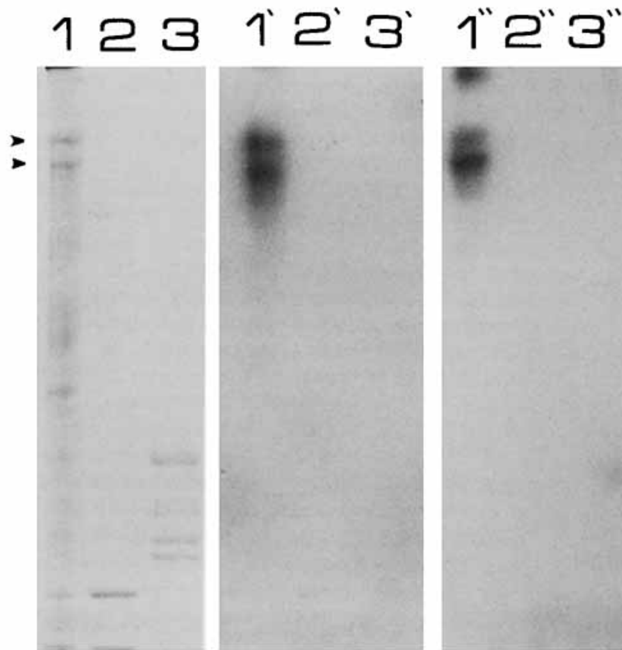

Fig. 7. Immunoblotting analysis of purified bovine desmosomes with antibody KM 4.62 and with monoclonal anti-desmoplakin DP1 and 2,2.15. 1–3, Coomassie blue stained bands in a matching gel; 1′–3′, autoradiogram of the immunoblot labeled with monoclonal anti-desmoplakin antibodies. Notice the positive and exclusive reactivity of antibody KM 4.62 with the two desmoplakin polypeptides (arrowheads) and the absence of labeling from other desmosomal elements or from the residual cytokeratins present in the specimen.

antibody KM 4.62 in contrast to the acini, which were essentially negative. Antibody KM 4.62 was also found to be very useful for the labeling of squamous metaplasia and neoplasia. The investigation of the latter two aspects is now in progress.

Additional information provided in the present study concerns the intracellular distribution of the 40-kD cytokeratin polypeptide. This aspect was investigated mostly by double immunofluorescence labeling of human cultured cells by antibody KM 4.62 and various polyclonal anti-keratins. The results of such experiments clearly indicated that, whenever the 40-kD protein was present, it was homogenously distributed throughout the entire cytokeratin network. In particular, there was no apparent enrichment of labeling near desmosomal junctions.

The second aspect to be discussed is related to the antigenic cross-reactivity observed between the human 40-kD cytokeratin and bovine desmoplakins. Obviously, the significance of this observation depends strictly on the specificity of our analysis, and several lines of evidence seem to support our conclusions: (1) KM 4.62 antibodies stained brightly punctuate structures in cells and tissues, which were identified as desmosomes owing to their intense labeling with different desmoplakin antibodies. (Attempts to immunolocalize the epitope at the electron microscope level have so far been frustrated by the apparent sensitivity of the epitope to aldehyde fixation.) (2) Desmosomes of all bovine epithelia tested were positively labeled with antibody KM 4.62, including stratified, pseudostratified, transitional, and simple, in line with the wide occurrence of at least desmoplakin I in all epithelial tissues [Moll and Franke, 1982; Franke et al, 1981b; Mueller and Franke, 1983; Cohen et al, 1983; Cowin et al, 1985]. (3) Immunoblotting analysis with isolated bovine desmosomes or purified desmosomal components directly demonstrated the reactivity of antibody KM 4.62 with desmoplakins I and II. Similar analyses performed with human tissues did not point to any reactivity of antibodies KM 4.62 with human desmoplakins, but to a rather exclusive reactivity with the 40-kD cytokeratin, whenever present.

The cross-reactivity detected by the particular antibody described here does not provide sufficient information to indicate the extent of structural homology between the human 40-kD cytokeratin and desmoplakins and to determine the molecular basis for this relationship. Our results, however, strongly suggest that some definitive structural homology does exist between the two, which is fortuitously revealed in the human-bovine system by antibody KM 4.62. This conclusion is further indirectly supported by some similarities between the amino acid compositions of desmoplakins and cytokeratins [Kapprell et al, 1985]. Obviously, a more comprehensive determination of the extent of structural homology between cytokeratins and desmoplakins will require both additional immunochemical analyses as well as detailed amino acid sequence data.

While it may be still premature to evaluate the biological significance of the apparent antigenic interrelationships between bovine desmoplakins and a human cytokeratin, it is reminiscent of one of the general features of the intermediate filament family, namely, the evolutionary conservation of certain regions concomitant with a remarkable diversification of other areas along the molecules. An example showing a similar phenomenon concerns the high molecular weight polypeptides of neurofilaments (the 160-kD polypeptides), which have been recently shown to contain amino-acid sequences typical of intermediate filaments [Geisler et al, 1985]. From the functional point of view, it is intriguing whether a structural homology between desmoplakins and cytokeratins reflects in any way the capacity of the former to interact with tonofilaments and anchor them to the membrane in desmosomal junctions. (For further discussion of cytokeratin-desmoplakin interaction see Jones and Goldman [1985] and Cowin et al [1985].) Additional studies along the lines discussed above will be necessary to directly assess this possibility.

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