

Immunological Comparison of Desmosomal Components From Several Bovine Tissues

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A panel of monoclonal antibodies and conventional antisera directed against desmosomal proteins from bovine muzzle epidermis was used to identify immunologically related proteins from two other bovine stratified squamous epithelia, cornea and esophagus. Desmosome-enriched tissue fractions were prepared from epidermis, cornea, and esophagus. These tissue extracts were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels, blotted onto nitrocellulose paper, and labeled using an indirect immunoperoxidase technique. Labeling with the conventional antisera demonstrates that each of the previously characterized epidermal desmosomal proteins or protein families has an immunologically cross-reacting counterpart in cornea and esophagus. However, chemical differences between homologous desmosomal proteins in these three tissues have also been detected. The corresponding proteins in the different tissues have similar but not always identical apparent molecular weights. Moreover, tissue-restricted antigenic determinants were detected in two of the desmosomal protein families using four monoclonal antibodies, each of which recognizes a distinct antigenic determinant.

Key words: desmosome, immunological analysis, immunoblotting

The nomenclature used to identify intercellular junctions has been based on ultrastructural criteria [1,2]. One class of junction, called the spot desmosome or macula adherens, has been described in a wide variety of epithelia and in the intercalated disc region of cardiac muscle. Although spot desmosomes from different tissues are ultrastructurally similar, the degree of similarity of their components has, until recently, not been examined.

Investigations of the biochemical composition of the spot desmosome have focused on the junctions of the bovine epidermis [3-8]. Through the use of immuno-

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electron microscopic labeling procedures, five biochemically characterized, immunologically distinguishable protein families have been localized to the desmosome. The term "protein family" refers to a set of proteins that have been determined to be closely related from direct structural comparisons and/or immunological cross-reactivity [9]. Two of the desmosomal protein families have been shown to be restricted to the intracellular plaque region: desmoplakin I/II [10-12], and an 81-kilodalton (kd) nonglycosylated protein, "desmoplakin III" [11-13]. Three glycoprotein families have been shown to contain determinants present in the intercellular zone of the desmosome: desmogleins I and II [11,12] and desmoglein III (Shida, Cohen, and Steinberg, unpublished observation). The term "desmoglein" refers to the presumed adhesive function of these proteins (-glea = glue) [14]. A sixth, and apparently minor, species-restricted desmosomal antigen, D-1, has been localized to the plaque, but its biochemical characterization is as yet incomplete [7,15]. Table I contains a list of the desmosomal proteins analyzed in this report and an explanation of the nomenclature used.

Tissue-to-tissue biochemical differences among desmosomes were first suggested by the observation of Borysenko and Revel [16] that desmosomes from different epithelial types differ in their sensitivity to EDTA, trypsin, and deoxycholate (DOC). The intercellular components of desmosomes from stratified squamous and simple cuboidal epithelia were dissociated by treatment with trypsin, DOC, or both but were unaffected by EDTA treatment. Conversely, desmosomes from simple columnar epithelia were disrupted by EDTA but not by trypsin or DOC.

Evidence of functionally distinguishable classes of desmosomes came from studies by Overton [17] and Overton and Kapmarsky [18]. These authors dissociated cells from different epithelial tissues, allowed them to reaggregate, and monitored desmosome formation by transmission electron microscopy. Embryonic chick corneal epithelial cells and embryonic mouse epidermal cells cooperated in the assembly of well-formed, hybrid desmosomes, but none of the other heterotypic cell combinations tested did so. Although adhesive selectivity is one explanation for these results, Overton [17] cautions that other possible explanations have not been completely ruled out. Armstrong [19] reported that "desmosomes" were formed at heterotypic cell borders in reaggregates containing embryonic chick heart and pigmented retina cells, an observation that has been discussed by Overton and Kapmarsky [18].

The biochemical differences suggested by the above experiments have not been detected by previous immunological studies [7,10,15,20,21]. Franke and co-workers [7,10,21] have shown by indirect immunofluorescent labeling of tissue sections, by immunoelectron microscopic labeling, and by immunoblotting experiments that polyclonal antisera to the closely related high molecular weight desmosomal plaque proteins (desmoplakins I and II) label a variety of bovine tissues and human tumors of epithelial origin. Franke et al [15] have reported that a guinea pig antiserum against the desmosomal plaque antigen D1 labels a variety of bovine tissues. Using indirect immunofluorescence, Cowin and Garrod [20] have observed that monospecific polyclonal antisera raised against all of the known desmosomal protein families label a variety of tissues in a wide range of species.

We report here the results of a biochemically oriented examination of the desmosomal components from a group of closely related bovine epithelia. Our assay entailed preparing desmosome-enriched fractions from three stratified squamous epithelia, resolving them on SDS-polyacrylamide gels, and comparing the protein patterns generated on immunoblots using a set of monospecific polyclonal antisera and

TABLE I. Nomenclature for Desmosomal Proteins From Bovine Muzzle Epidermis (M_r in kilodaltons)*

Proposed designations	Designations from reference 7 ^a	Designations from reference 20
Desmoplakin I (DP I, 240)	Desmoplakin I (250)	Group E (230)
Desmoplakin II (DP II, 210)	Desmoplakin II (215)	(205)
Desmoglein Ia (DG Ia, 155)	Group 3 (< 180)	Group D (150, triplet)
Desmoglein Ib (DG Ib, 150)		
Desmoglein Ic (DG Ic, 145)		
Desmoglein IIa (DG IIa, 118)	Band 4a (< 130)	Group C (115, doublet)
Desmoglein IIb (DG IIb, 97)	Band 4b (> 115)	
Desmoplakin III (DP III, 81)	Band 5 (83)	Group B ^a {
Desmoplakin IV (DP IV, 77) ^b	Band 6 (75)	
Desmoglein III (DG III, 22)		(82)
		Group A (22)

*Explanation of the proposed nomenclature. The first element (DP or DG) indicates desmoplakin (used here to designate any plaque-restricted protein) or desmoglein. The second element, a Roman numeral (I–II), indicates the protein family. (Tissue-to-tissue molecular weight variations (see below) argue against incorporating references to molecular weight into these designations.) An optional third element, a lowercase letter (a–c), assigned in order of decreasing M_r , identifies the individual family member. Additional qualifying descriptions may be added as necessary, eg, “bovine epidermal DG IIa.” Because the designations “desmoplakins I and II” have already been used in reference to the 240-kd and 210-kd plaque proteins [7,10], which are members of a single protein family, we retain these designations but refer to this protein family as DP I/II.

^aCowin and Garrod [20] have grouped together the 86- and 82-kd proteins based on an apparent immunological relatedness. However, Franke et al [8] have shown that these two proteins are structurally quite dissimilar, and we provide evidence in this paper that these two proteins (DP III and DP IV) are not closely related immunologically (Fig. 2).

^bA 77-kd protein is present in isolated desmosome preparations but has not yet been immunologically localized within the desmosome. This protein is nonglycosylated and separates from the desmosome core under conditions which also separate DP I/II and DP III from the core [6]. Since this protein has properties indicative of a plaque component, we have provisionally designated it as desmoplakin IV.

monoclonal antibodies. This method allows one to calculate and compare the apparent molecular weights of immunologically cross-reactive antigens and to determine the relative enrichment of these antigens in different fractions. The use of monoclonal antibodies is an important tool in the detection of tissue-specific antigenic determinants not detectable with polyclonal antibodies.

MATERIALS AND METHODS

Preparation of Desmosome-Enriched Tissue Fractions

Desmosome-enriched fractions were prepared from stratum spinosum of bovine muzzle, bovine corneal epithelium, and bovine esophagus following the procedure of Gorbsky and Steinberg [6] as modified by Gorbsky (unpublished). Fifteen grams of minced tissue was extracted for 3 hrs at 4°C in 500 ml of 0.05% Nonidet P-40 (NP-

40), 5 $\mu\text{g/ml}$ pepstatin (Sigma Chemical Co., St. Louis, MO) in 0.1 M citric acid:sodium citrate buffer pH 2.6 (CASC A). The large tissue fragments were removed by filtering the suspension through 51- μm mesh polyester netting. The filtrate was centrifuged at 13,000g for 20 min and the supernatant was discarded. The pellet was resuspended in 80 ml of 0.01% NP-40 in 0.1 M citrate buffer with pepstatin, pH 2.6 (CASC B), and sonicated in a Heat Systems W-220F sonicator at setting 7 for ten periods of 15 sec each, with 10-sec rest intervals. The sonicated desmosome fraction was centrifuged at 750g for 20 min and the pellet was discarded. Twenty milliliters of supernatant was added to each of four 40-ml centrifuge tubes, underlayered with 10 ml of 50% sucrose in CASC B (w/v), and centrifuged at 12,000g for 20 min at 4°C. Desmosomes collect at the interface between the supernatant and the sucrose cushion. This band was collected and washed three times in 80 ml of CASC B by centrifugation at 12,000g for 20 min.

Preparation of Antibodies

Polyclonal antibodies were raised in rabbits by immunization with desmosomal proteins that were purified in the following manner. Bovine epidermal desmosomal proteins were separated on a 5–20% polyacrylamide gradient gel, stained with Coomassie blue, destained, and individual desmosomal proteins or protein families (as determined by Cohen et al [9, 22] were sliced out. The gel slices were neutralized in phosphate-buffered saline (PBS) and finely ground in a Potter-Elvehjem homogenizer. A volume of acrylamide homogenate containing approximately 200 μg of protein was mixed with an equal volume of complete Freund adjuvant and injected subcutaneously and intramuscularly into rabbits. Subsequent subcutaneous injections at 2–4-week intervals contained the same amount of protein-acrylamide homogenate mixed with incomplete Freund adjuvant.

Two of the resulting antisera, R_pDG I-1 and R_pDG II-1, have been described elsewhere ([22]; see Figure 1 for an explanation of our antibody nomenclature). Antisera R_pDP III-1, R_pDG II-1, and R_pDG II-2 reacted not only with the appropriate gel-purified immunogen, but also showed weak labeling of the 150 kd triplet desmoglein I. These cross-reactivities were eliminated by incubating the three antisera with slices of nitrocellulose blots containing desmoglein I.

The preparation of the monoclonal antibodies used here has been described previously [9]. However, the system of nomenclature used here to designate antibody-

TABLE II. Binding of Monospecific Polyclonal Antibodies to Desmosomal Components on Immunoblots*

Antibody designation ^a	M_r of target antigen (kd)	Muzzle epidermis	Cornea	Esophagus
R _p DP I/II-1	240/210	+	+	+
R _p DP III-1	81	+	+	+
R _p DG I-1	155–145 ^b	+	+	+
R _p DG II-1	118–97 ^c	+	±	±
R _p DG II-2	118–97 ^c	+	±	±

*+, positive; ±, very weak.

^aSee Figure 1.

^b M_r range of corneal antigens is slightly higher.

^c M_r of corneal and esophageal DG IIb is slightly lower than 97 kd.

ies (Figure 1) differs from that used previously. Our designations for the desmosomal protein families and the antibodies directed against them are presented in Tables I-III.

Indirect Immunofluorescent Labeling of Tissue Sections

Small pieces of bovine muzzle epidermis, cornea, and esophagus were embedded in OCT embedding medium (Fisher) and sectioned at -18°C on a Slee cryostat. The sections were collected onto gelatin-coated slides, air-dried, fixed in 70% ethanol, and rehydrated in PBS, pH 7.4, for 1 hr. The sections were pre-incubated in 2%

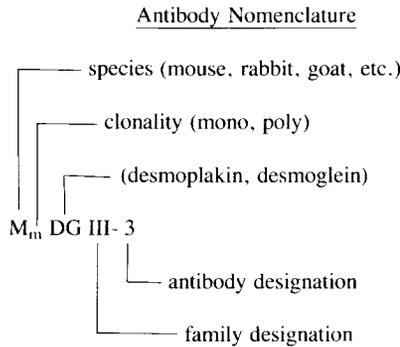


Fig. 1. Explanation of antibody nomenclature. The system we adopt here may prove to be generally convenient. The first element indicates the English common name of the species in which the antibody was raised (R = rabbit, M = mouse, RT = rat, G = goat, GP = guinea pig, H = human). This is followed by a subscript (p or m) to denote whether the antibody is polyclonal or monoclonal. Next comes the designation of the immunogen(s) or antigenic target(s). Last comes a hyphenated Arabic numeral to distinguish between two or more antibodies directed against the same antigenic target. Thus, the third in a series of mouse monoclonal antibodies directed against the lowest molecular weight bovine desmosomal core glycoprotein would be designated M_mDG III-3.

TABLE III. Binding of Monoclonal Antibodies to Desmosomal Components on Immunoblots*

Previous anti-body designation [9, 22]	New antibody designation	M _r of epidermal target antigen (kd)	Epidermis	Cornea	Esophagus
B1C2-1	M _m DP I-1	240	+	+	+
B1A1-2	M _m DP I/II-1	240/210	+	+	+
A3D3-3	M _m DG I-1	155-145	+	+	+
A16E1-2	M _m DG I-2	"	+	+	+
A15A5-1	M _m DG I-3	"	+	+	+
A1A3-2	M _m DG I-4	"	+	±	±
A10C6-1	M _m DG I-5	"	+	+	+
AU-1	M _m DG I-6	"	+	+	+
A2F4-1	M _m DG II-1	118-97	+	-	-
A16C2-2	M _m DG II-2	"	+	-	-
A15D3-1	M _m DG II-3	"	+	-	-
A17D6-3	M _m DG II-4	"	+	+	+
A2A3-1	M _m DG III-1	22	+	+	+

*+, positive; ±, very weak; -, negative.

bovine serum albumin (BSA) in PBS at room temperature for 30 min, then incubated in antidesmosomal antibody for 45 min. The polyclonal rabbit antisera were used at a 1:50 dilution in 2% BSA in PBS. The monoclonal antibodies were used as undiluted hybridoma tissue culture supernatants. Antibody-labeled sections were washed in PBS, incubated in a 1:40 dilution of second antibody for 30 minutes and washed again with PBS. Fluorescein isothiocyanate (FITC)-conjugated goat antirabbit IgG (Cappel) was used to detect the polyclonal antisera. FITC-conjugated goat antimouse IgG (Cappel) was used to detect the monoclonal antibodies. The sections were examined on a Zeiss Universal microscope equipped with epifluorescence accessories.

Indirect Immunoperoxidase Labeling of Nitrocellulose Blots

Desmosome-enriched tissue fractions were electrophoresed on 5–20% SDS-polyacrylamide gels according to the procedure of Laemmli [23]. The proteins were electrophoretically transferred to nitrocellulose paper as described previously [9,24]. The nitrocellulose blots were preincubated in 3% BSA-PBS, pH 7.4, at room temperature for 1 hr, then incubated in the antidesmosomal antibody for 2 hrs. Monoclonal antibodies were used as undiluted hybridoma tissue culture supernatants. Polyclonal antisera were diluted 1:100 or 1:250 in 3% BSA-PBS, pH 7.4. After six 5-min washes in PBS, the blots were incubated in either peroxidase-conjugated sheep antimouse IgG (Cappel) diluted 1:500 in 3% BSA-PBS or in peroxidase-conjugated goat anti-rabbit IgG (Cappel) diluted 1:500 in 3% BSA-PBS as appropriate. Blots were washed again in PBS, and the peroxidase reaction was developed with diaminobenzidine (0.5 mg/ml) in 0.05 M Tris-HCl, pH 7.4, with 0.03% H₂O₂.

RESULTS

Immunoblotting

Western blots of desmosome-enriched fractions from epidermis, cornea, and esophagus labeled with a representative set of antidesmosomal antibodies are shown in Figures 2,3. Since relative amounts of nondesmosomal proteins varied in the three preparations, loadings were not determined by total protein. The gels were loaded such that the three tissue extracts showed approximately equal intensities of label with polyclonal antiserum, R_pDG I-1. Under these conditions, four of the five monospecific polyclonal antisera and nine of the ten cross-reacting monoclonal antibodies labeled all three tissue extracts with very similar intensities, supporting the validity of this method for determining loadings.

The corresponding desmosomal components from the three tissues are immunologically cross-reactive with all of the polyclonal antisera (Table II) and certain of the monoclonal antibodies (Table III). The corresponding proteins have very similar apparent molecular weights (Figs. 2,3) and were extracted under similar conditions. However, even among these histologically similar epithelia, differences between the corresponding desmosomal glycoprotein components were discerned.

Both major desmosomal core glycoprotein families, DG I and DG II, exhibit tissue-to-tissue differences both in apparent molecular weight and in immunological reactivity. M_mDG I-4 shows only a very weak cross-reaction with its target antigens in cornea and esophagus (Fig. 3). Three monoclonal antibodies directed against three distinct antigenic determinants on epidermal DG II (as described by Cohen et al [9]) do not react with DG II from cornea or esophagus (see Table III). In an attempt to

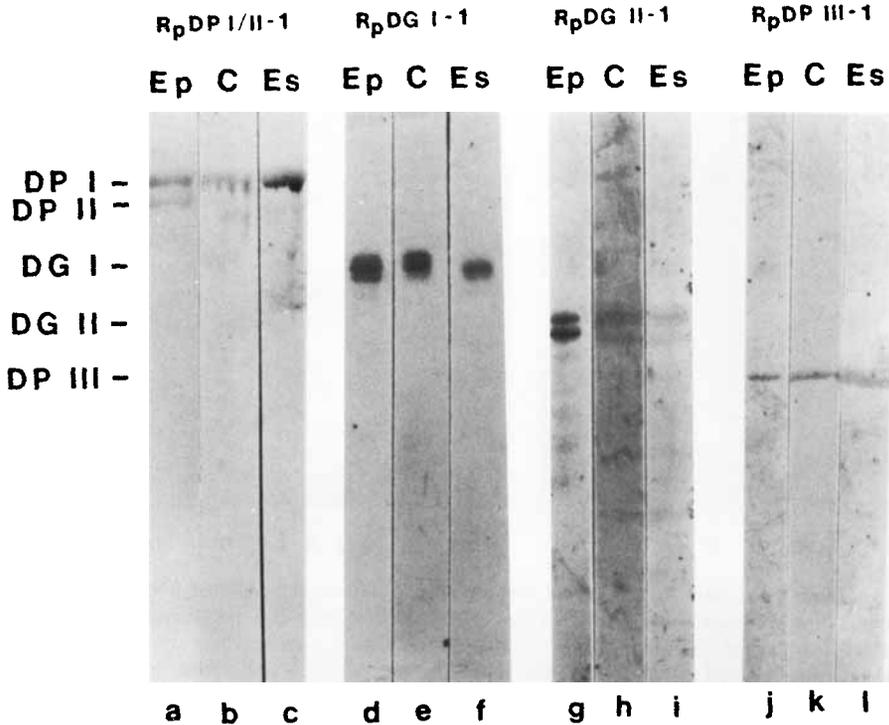


Fig. 2. Polyclonal antibody labeling of immunoblots of desmosome-enriched fractions from bovine epidermis (Ep), cornea (C), and esophagus (Es) electrophoresed on 5–20% polyacrylamide gels. The major proteins from cornea and esophagus that react with R_pDP I/II-1 comigrate with epidermal DP I (lanes a–c). Corneal proteins that react with R_pDG I-1 have slightly higher M_r 's relative to desmoglein I of epidermis and esophagus (lanes d–f). Proteins of similar M_r from all three tissue extracts react with R_pDG II-1; however, the staining intensity of both the corneal and esophageal protein bands is very weak (lanes g–i). Extracts from all three tissues show a single 81-kd immunoreactive band on blots labeled with R_pDP III-1 (lanes j–l).

detect possible weak labeling of DG II by M_mDG II-1 in cornea or esophagus, twice as much desmosomal protein was loaded in lanes k, l, n, and o in Figure 3, as was loaded in every other lane. M_mDG II-4 labeled corneal and esophageal DG II strongly, whereas M_mDG II-1 showed no labeling in the cornea or esophagus lanes. Even R_pDG II-1, the polyclonal antiserum against epidermal DG II, showed a low level of labeling of corneal and esophageal DG II (see Fig. 2).

Immunohistochemical Labeling

A representative set of the monoclonal antibodies that were used in the immunoblotting experiments were also used to label cryostat sections of bovine epidermis, cornea, and esophagus. Those antibodies that reacted on immunoblots with a desmosomal protein extracted from a particular tissue also exhibited a desmosomelike labeling pattern on frozen sections of that tissue. Specifically, M_mDP I/II-1, M_mDG I-1, M_mDG II-4, and M_mDG III-1 reacted with a cell surface component showing a punctate distribution in the epithelial layer of all three tissues. M_mDG II-1, which recognized a desmosomal protein on immunoblots of extracts of epidermis but not of

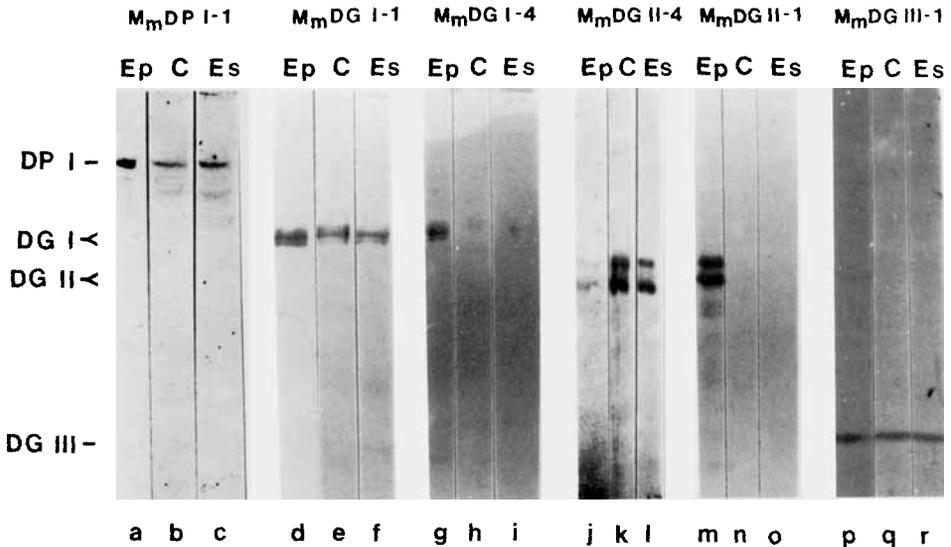


Fig. 3. Monoclonal antibody labeling of immunoblots of desmosome-enriched fractions from bovine epidermis (Ep), cornea (C), and esophagus (Es) electrophoresed on 5–20% polyacrylamide gels. M_m DP I-1 (lanes a–c) labeled a single band ($M_r = 240$ kd) in all three tissues. The lower molecular weight immunoreactive material in lanes b and c does not comigrate with DP II and is presumed to be proteolytic breakdown products. M_m DG I-4 (lanes g–i) and M_m DG II-1 (lanes m–o) discriminated epidermal DG I and DG II, respectively, from their corneal and esophageal homologues. Lanes k, l, n, and o were loaded with twice as much desmosome-enriched material as all other lanes to emphasize the absence of antibody reaction in lanes n and o. The other antidesmosomal monoclonal antibodies depicted did not discriminate among target antigens from these three tissues.

cornea or esophagus, showed the same tissue specificity in the labeling of frozen sections (see Fig. 4).

DISCUSSION

Immunoperoxidase labeling patterns on nitrocellulose blots generated by our monospecific polyclonal antisera and one subset of our monoclonal antibodies demonstrate that five major desmosomal protein families from bovine epidermis, DP I/II, DP III, DG I, DG II, and DG III, have immunologically cross-reactive counterparts of similar apparent molecular weights in bovine cornea and esophagus. However, a different subset of our monoclonal antibodies reveals one antigenic site on epidermal DG I and three distinct sites on epidermal DG II that are either absent from or have been altered in the corresponding protein families in cornea and esophagus.

R_p DP III-1 and R_p DG I-1 polyclonal antisera exhibited complete cross-reaction on immunoblots with all three tissue extracts. However, two other polyclonal antisera, R_p DG II-1 and R_p DG II-2, labeled corneal and esophageal desmoglein II less intensely than epidermal desmoglein II (see Fig. 2, lanes g,h,i; (Table II)). The reduction in intensity of label could in principle result either from antigenic differences between DG II proteins in the three tissues or from smaller amounts of DG II in the cornea and esophagus preparations. However, M_m DG II-4 labels all three tissue preparations

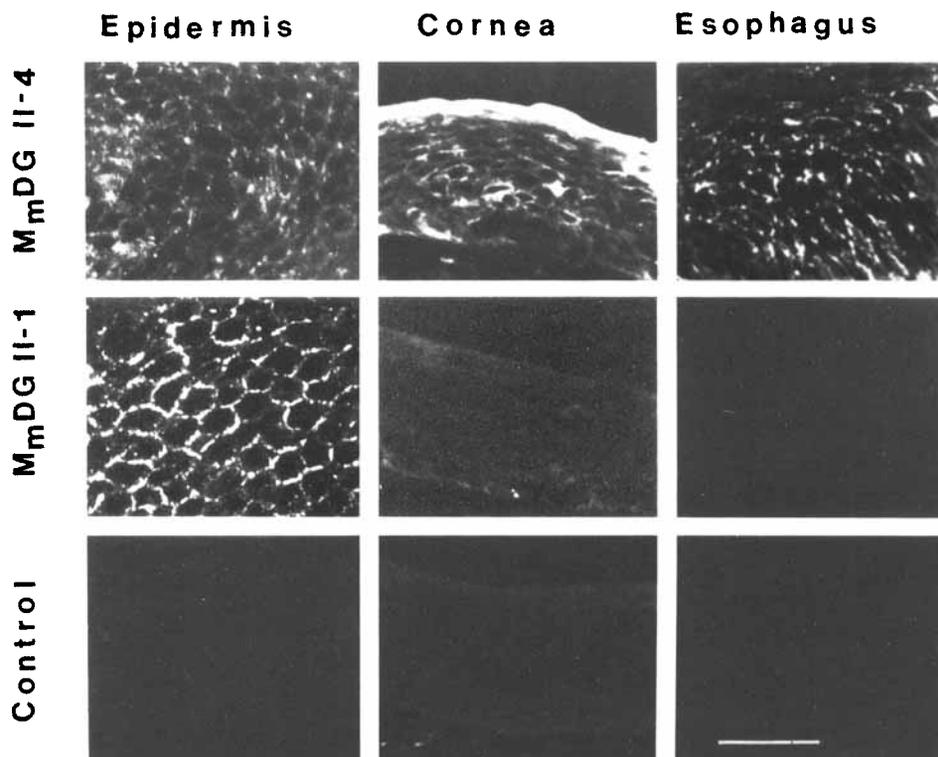


Fig. 4. Indirect immunofluorescent labeling of frozen sections of bovine epidermis, cornea and esophagus using monoclonal antibodies M_m DG II-4, M_m DG II-1, and a control monoclonal antibody directed against an embryonic chick neural retina cell surface antigen (provided by Dr. William Thomas). M_m DG II-4 shows cell surface labeling in all three tissues, whereas M_m DG II-1 shows labeling only in epidermis, in agreement with the immunoblotting results shown in Figure 2. The control antibody demonstrates the background level of fluorescence. Bar = 50 μ m.

with approximately equal intensity. Moreover, the other three monoclonal antibodies to epidermal DG II do not cross-react at all with corneal or esophageal DG II. Taken together, these results suggest that the labeling pattern obtained with R_p DG II-1 is due to antigenic differences between DG II proteins from the different sources.

Using two polyclonal antibodies against desmoglein II, Cowin and Garrod [20] observed a reduction in fluorescent labeling intensity on sections of heart, liver and gut relative to the labeling intensity seen in epidermis. The tissue-to-tissue antigenic differences in desmoglein II revealed by our immunoblotting experiments may account for these observations.

R_p DP I/II-1, a polyclonal antiserum that reacts with both desmoplakin I and II from epidermis, shows significant labeling of only a single band in the cornea and esophagus extracts comigrating with epidermal DP I (Fig. 2, lanes a,b,c). Mueller and Franke [7] have previously shown epidermal desmoplakins I and II to be closely related based on peptide mapping and immunological cross-reactivity. M_m DP I-1 is a monoclonal antibody that distinguishes between these two proteins, binding to epidermal DP I but not to epidermal DP II. On immunoblots, M_m DP I-1 labels the same corneal and esophageal antigen(s) as R_p DP I/II-1 (Fig. 3, lanes a,b,c). Based on the

above information it appears that DP I, but not DP II, is present in cornea and esophagus; however, we have not ruled out the possibility that corneal and/or esophageal DP II were lost during the extraction procedure. In a related study, Franke et al [10] reported that a polyclonal antiserum directed against DP I/II, applied to immunoblots, labeled a band from esophageal and myocardial extracts that comigrates with epidermal DP I. Little or no DP II was detected in those extracts.

The method of comparing desmosomal components from different tissues through the use of immunoblots complements previous immunohistochemical studies [10,20,21]. While the immunohistochemical approach reveals the histological location of immunologically related antigens, our immunobiochemical approach reveals the apparent molecular weights of these antigens, identifies differences between them and can be used to study their biochemical properties (eg, solubilities and sensitivity to proteases).

Desmoglein I and desmoglein II can be added to the growing list of functionally related proteins found to differ biochemically from tissue to tissue. Other pertinent examples include the gap junction and intermediate filament proteins. The major proteins of gap junctions from lens, liver and heart tissue, while presumed to be functionally related, appear from peptide mapping and immunological data [25-27] to be biochemically distinct. Different classes of intermediate filaments are composed of immunologically distinguishable proteins [28,29] with common structural features [30-33].

We are interested in investigating the functional significance of the tissue-specific antigenic determinants in epidermal desmogleins I and II that we have described here. One possibility is that one or more of these tissue-specific determinants may be part of a domain involved in protein-protein interactions. Such studies may elucidate the biochemical basis for the selectivity in desmosomally mediated cell-cell recognition suggested by the work of Overton [17].

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NOTE ADDED IN PROOF

Cowin et al (*J Cell Sci* 66:119, 1984) have recently reported the preparation of an anti-serum which reacts with the 86 K but not the 82 K protein.