Correlation of Specific Keratins with Different Types of Epithelial Differentiation: Monoclonal Antibody Studies

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Summary

We have prepared three monoclonal antibodies against human epidermal keratins. These antibodies were highly specific for keratins and, in combination, recognized all major epidermal keratins of several mammalian species. We have used these antibodies to study the tissue distribution of epidermis-related keratins. In various mammalian epithelia, the antibodies recognize seven classes of keratins defined by their immunological reactivity and size. The 40, 46 and 52 kilodalton (kd) keratin classes were present in almost all epithelia; the 50 kd and 58 kd keratin classes were detected in all stratified squamous epithelia, but not in any simple epithelia; and the 56 kd and 65–67 kd keratin classes were unique to keratinized epidermis. Thus the expression of specific keratin classes appeared to correlate with different types of epithelial differentiation (simple versus stratified; keratinized versus nonkeratinized).

Introduction

Keratins are a family of water-insoluble proteins of 40–70 kd, classically thought to form the intermediate filaments (10 nanometer diameter) of only the epidermis and its appendages (Tezuka and Freedberg, 1972; Baden et al., 1973; Skerrow et al., 1973; Shimizu et al., 1974; Steinert and Idler, 1975; Huang et al., 1975; Dale et al., 1976; Brysk et al., 1977; Drochmans et al., 1978; Sun and Green, 1978a; Steinert et al., 1979). More recent studies have established that such filaments are present in almost all epithelia, both in vivo and in culture (Franke et al., 1978a, 1978b, 1979a; Sun and Green, 1978b; Sun et al., 1979). Keratin filaments have also been shown to be related to, but immunologically distinguishable from, other types of intermediate filaments, including vimentin filaments of mesenchymal cells, desmin filaments of muscle cells, neurofilaments of neuronal cells and glial filaments of astrocytes (for review, see Lazarides, 1980).

The subunit composition of keratin filaments is extremely variable. Different keratin patterns have been found in different epithelia (Doran et al., 1980; Fuchs and Green, 1980; Gipson and Anderson, 1980; Franke et al., 1981a, 1981b, 1981c; Milstone and McGuire, 1981). Moreover, even within a given epithelium, keratin composition varies with anatomical location (Drochmans et al., 1978; Lee et al., 1979), cellular growth environment (Sun and Green 1978a; Fuchs and Green, 1978; Shimizu et al., 1974; Steinert and Yuspa, 1978; Kubilus et al., 1979; Radian et al., 1980; Doran et al., 1980; Fusenig et al., 1981), stage of differentiation (Fuchs and Green, 1980, Sun et al., 1982; Woodcock-Mitchell et al., 1982) and period of embryonic development (Beckingham-Smith, 1973; Dale et al., 1976). The biological significance of such striking heterogeneity in keratin filament composition is obscure.

To elucidate the functional significance of various keratin polypeptides, we have investigated the cell and tissue distribution of several keratin antigens using three monoclonal antibodies to keratins, designated AE1, AE2 and AE3. These antibodies are highly specific for keratins and, in combination, recognize all major human epidermal keratins (Woodcock-Mitchell et al., 1982). Thus they provide specific and sensitive probes for detecting epidermis-related keratins in various epithelia.

We report that keratins of mammalian epithelia can be divided into seven classes according to their immunological reactivity and size. The expression of specific keratins appears to correlate with the type of epithelial differentiation (simple versus stratified; keratinized versus nonkeratinized). We also show that, among keratins with common antigenic determinants, the smaller ones are expressed in almost all epithelia, whereas the larger ones are expressed only in more complex or specialized epithelia.

Results

Specificity of the Antibodies: Immunofluorescence Microscopy

The three monoclonal antibodies used, AE1, AE2 and AE3, were produced by hybridoma cells resulting from the fusion between P3X63 Ag8 myeloma cells (P3 cells) and spleen cells of BALB/c mice immunized with human epidermal keratins. All three antibodies decorate specifically keratin fibers in cultured human epidermal cells (Woodcock-Mitchell et al., 1982). To determine their cross-reactivity with keratins of epithelia other than epidermis, we used these antibodies to stain various cultured cells and monkey tissue sections by the indirect immunofluorescence technique.

AE1 antibody decorated a network of cytoplasmic fibers characteristic of keratins in many cultured epithelial cells. These included primary cultures of monkey epidermal cells (Figure 1a); rabbit epidermal cells...
Figure 1. Immunofluorescence Staining of Cultured Cells with AE1 Monoclonal Antibody

Cells were grown on 12 mm glass coverslips, fixed with methanol and stained with AE1 by indirect immunofluorescence. Note the staining of keratin fibers in various epithelial cells.

(a) Monkey skin epidermal cells (primary culture). Arrows indicate cell-cell junctions presumably containing desmosomes (Sun and Green, 1976b). Some epidermal cells stained more intensely than others. (b) Rabbit epidermal cells (primary culture). (c) A rabbit conjunctival epithelial cell (primary culture). (d) A rabbit thymic epithelial cell (primary culture). (e) A multinucleated human corneal epithelial cell (secondary culture). (f) Human bladder epithelial cells (primary culture). Note the pronounced perinuclear ring of keratin fibers in this cell type. (g) HeLa cells. Note the staining in some cells of fibers radiating from a perinuclear site. Also note that some cells (asterisks) were stained only weakly by AE1 antibody (although all cells stained positively with antisera to total human epidermal keratins or with AE3 antibody; see Figure 3b; also see Franke et al., 1979b). (h) PtK2 cells. (i) Human fibroblasts (WI-38). Note the absence of staining. Bar = 25 μm.

(Figure 1b), esophageal epithelial cells, corneal epithelial cells, conjunctival epithelial cells (Figure 1c) and thymic epithelial cells (Figure 1d); and human corneal epithelial cells (Figure 1e) and bladder epithelial cells (Figure 1f). Similar staining of keratin fibers was observed in several permanent epithelial cell lines, including human ME-180 cells, human SCC-12 cells (Wu and Rhoenwald, 1981), rat kangaroo PtK2 cells (Figure 1b) and a subpopulation of HeLa cells (Figure 1g; see Franke et al., 1979b). No staining was observed in human neuroblastoma cells (IMR-32), human fibroblasts (WI-38; Figure 1i) or several other nonepithelial cell types. Control experiments showed that medium conditioned by parent P3 myeloma cells did not produce significant staining in any of the aforementioned cell types. These results indicated
that AE1 antibody recognized keratin fibers in a wide variety of cultured epithelial cells and that the antigenic determinant was conserved in keratins of several mammalian species.

In frozen sections of monkey tissues, AE1 antibody stained all epithelia tested, including those of bladder (Figure 2a), gall bladder, thymus (Figure 2b), and pancreatic ducts (Figure 2c). Like conventional anti-keratin antisera, AE1 antibody failed to stain parenchymal cells of pancreas or liver (Sun et al., 1979; Franke et al., 1979a, 1981a). Interestingly, it stained selectively the basal layer of both epidermis and esophageal epithelium (Figure 2d), suggesting that at least one of the keratins recognized by this antibody must be expressed by basal cells (Woodcock-Mitchell et al., 1982).

AE2 antibody stained weakly keratin fibers in cultured human epidermal cells and SCC-12 human squamous carcinoma cells (Figure 3a), but produced no staining in HeLa (Figure 3b) or PtK2 cells. Furthermore, in tissue sections, this antibody stained suprabasal cells of the epidermis (Woodcock-Mitchell et al., 1982), but did not stain several other epithelia such as those of the esophagus, bladder and pancreas. These results suggested that keratin antigens specified by AE2 antibody had restricted tissue distribution (see below).

AE3 antibody decorated keratin fibers in many cultured epithelial cells, including SCC-12 cells (Figure 4a), HeLa cells (Figure 4b) and PtK2 cells (Figure 4c). No staining of fibroblasts or other nonepithelial cells was noted. In tissue sections, AE3 antibody stained uniformly all epithelia tested, including those of skin, esophagus, bladder and pancreas (ducts and parenchymal cells), with no detectable staining of mesenchymal cells (not shown). Thus, like AE1, AE3 antibody recognized keratins in a wide spectrum of epithelial cells.

Specificity of the Antibodies: Immunoblot Analysis
To establish the specificity of the monoclonal antibodies, we studied their binding to keratin polypeptides of human, monkey and rabbit epidermis by the immunoblot technique (Towbin et al., 1979; see Experimental Procedures). SDS-polyacrylamide gel electrophoresis showed that epidermal keratins of these three species were similar, each consisting of four major components (50, 56, 58 and 65-67 kd; Figure 5). Immunoblot experiments demonstrated that the major epidermal keratins of all three species were recognized by at least one of the three monoclonal antibodies: the 50 kd keratin was recognized by AE1, the 56 kd keratin by both AE1 and AE2, the 58 kd keratin by AE3 and the 65-67 kd keratins by both AE2 and AE3 (see below; also Woodcock-Mitchell et al., 1982). Thus the antigenic sequences recognized by the three antibodies were highly conserved in epidermal keratins of similar size in the three species. These results also established that, in combination, the three antibodies can be useful for detecting epidermis-related keratins in various human, monkey and rabbit epithelia.

Figure 2. Immunofluorescence Microscopy of Frozen Tissue Sections with AE1 Antibody
All tissues were from Cynomologus monkey. (a) Bladder. (b) Thymus. (c) Pancreas. (d) Esophagus. Note the staining of esophageal epithelial basal cells. Bar = 50 μm.

Figure 3. Immunofluorescence Staining of Cultured Cells with AE2 Antibody
(a) Human squamous carcinoma cell line SCC-12 (Wu and Rheinwald, 1981). (b) HeLa cells. Note the absence of staining. Bar = 25 μm.
Figure 5. Comparison of Epidermal Keratins from Human, Monkey and Rabbit

Water-insoluble proteins prepared from trunk epidermis of human (lane 1), monkey (lane 2) and rabbit (lane 3) were analyzed by SDS-polyacrylamide gel electrophoresis and stained with Coomassie blue. High molecular weight bands represent collagen contaminants that do not react with antikeratin antibodies. "a" denotes actin.

Figure 4. Immunofluorescence Staining of Cultured Cells with AE3 Antibody

(a) SCC-12 cells. (b) HeLa cells. Note that all cells were stained and the stained fibers appear to be distributed rather uniformly in the cells (see Figure 1g). (c) PK2 cells. Bar = 25 μm.

Tissue Distribution of Keratins

To investigate the tissue distribution of keratins, we analyzed keratins from a variety of epithelia representing different types of epithelial structure: keratinized epithelium (epidermis), nonkeratinized stratified squamous epithelia (cornea, conjunctiva, esophagus), pseudostratified columnar epithelium (trachea), transitional epithelium (bladder) and simple epithelia (gall bladder, stomach, intestine, kidney, pancreas, diaphragm-peritoneal mesothelium) (see Table 1).

Coomassie Blue Staining

Water-insoluble cytoskeletal proteins were prepared from a variety of human and monkey stratified squamous epithelia, analyzed by SDS-polyacrylamide gel electrophoresis and visualized by Coomassie blue staining (Figure 6). The 50 and 58 kd keratins were present, in variable amounts, in all stratified squamous epithelia. However, some keratins appeared to be tissue-specific. A 57 kd keratin was present in tongue (lane 6), esophagus (lane 7) and cervix (not shown), whereas a 55 kd keratin was found only in cornea (lane 3; Doran et al., 1980; Franke et al., 1981b). A 65–67 kd keratin component appeared to be skin-specific (lanes 1, 4 and 5), although a keratin in the same molecular weight range (65 kd) was detected in cornea (lane 3; Doran et al., 1980; Franke et al., 1981b). These findings are in agreement with previous results and provide further evidence that keratins are highly heterogeneous (Doran et al., 1980; Fuchs and Green, 1980; Gipson and Anderson, 1980; Franke et al., 1981a, 1981b, 1981c; Milstone and McGuire, 1981).
Table 1. Tissue Distribution of Epidermis-Related Keratins

<table>
<thead>
<tr>
<th>Keratin Class (kd)</th>
<th>Antibody</th>
<th>Keratinized*</th>
<th>Stratified*</th>
<th>Simple*</th>
<th>Possible Marker for</th>
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<td>-</td>
<td>Keratinization</td>
</tr>
<tr>
<td>52</td>
<td>AE3</td>
<td>-</td>
<td>+*</td>
<td>-</td>
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<td>-</td>
<td>+*</td>
<td>-</td>
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<tr>
<td>40</td>
<td>AE1</td>
<td>-</td>
<td>+</td>
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* Classified according to the histology of the major epithelial cell type.

Keratinized epithelium: epidermis.

Stratified, nonkeratinized epithelia: esophagus, cornea, conjunctiva, thymus, bladder (transitional), trachea (pseudo-stratified columnar).

Simple epithelia: gall bladder, stomach, small intestine, liver, kidney, pancreas, diaphragm.

The 58 and 50 kd keratin classes contain multiple keratin species expressed in a tissue- and differentiation-dependent fashion (see text).

Tentative identification.

Immunoblot Analysis

Monoclonal antibodies AE1, AE2 and AE3 were used to compare keratins of various epithelia by the immunoblot technique. Water-insoluble or total cellular proteins were prepared from various monkey and rabbit epithelia, separated on SDS-polyacrylamide gels and reacted with the antibodies as described. Since our major goal was to examine the possible correlation of keratin expression with epithelial differentiation, it was important to ensure that the detected keratins represented intact, undegraded molecules. Preliminary experiments showed that despite the inclusion of several protease inhibitors (PMSF, antipain, pepstatin, EDTA and EGTA) in the extraction buffer, keratins of some internal organs (particularly the digestive tract) frequently underwent slight proteolytic degradation. Although the degradation was not always obvious by Coomassie blue staining of the gels, additional low molecular weight bands were readily detected by the more sensitive peroxidase-antiperoxidase technique of antibody staining (see Franke et al., 1981c). Boiling these tissues in high concentrations of SDS and dithiothreitol (DTT), without prior extraction in aqueous buffer, minimized the problem of degradation and generated reproducible immunoblot results.

AE1 Antibody

In both human and monkey epidermis, AE1 antibody recognized a 56 kd band and two to three closely spaced bands of approximately 46-50 kd (Figure 7a, lanes 1 and 2). The 56 kd keratin appeared to be unique to the epidermis; it was only faintly detected in esophagus (lane 3), bladder (lane 8) and trachea (lane 9), and was virtually undetectable in all other epithelia. In contrast, the keratin bands of 46-50 kd (referred to as the 50 kd keratin class; Table 1) were found in all stratified squamous epithelia (lanes 1-7), as well as in epithelia of the bladder (lane 8) and trachea (lane 9), but not in any simple epithelia (lanes 10-13). In
addition, AE1 antibody detected a distinct 40 kDa band in all epithelia except keratinized epidermis (lanes 1 and 2). Coomassie blue staining of the gels indicated that this 40 kDa keratin was particularly abundant in conjunctival epithelium (Figure 6, lane 2; also see Fuchs and Green, 1981) and in some simple epithelia. AE1 antibody also detected a 37 kDa band of variable intensity in liver (Figure 7a, lane 12) and stomach (lane 13). Whether this band represented a separate keratin species or a degradation product was not clear.

Analysis of the rabbit tissues yielded almost identical results (Figure 7b). A 56 kDa keratin was detected mainly in epidermis (lanes 1 and 2), a 50 kDa keratin was detected in all stratified squamous epithelia (lanes 3 to 6) and pseud stratified tracheal epithelium (lane 7) and a 40 kDa keratin was detected in almost all epithelia.

**AE2 Antibody**

Concident with the immunofluorescence microscopic data, AE2 antibody appeared to be specific for some epidermal keratins. In the epidermis of human (Figure 8a, lane 1), monkey (Figure 8a, lane 2) and rabbit (Figure 8b, lanes 1 and 2), this antibody detected a 56 kDa keratin and 65–67 kDa keratins. These keratins were not found in any other epithelia. The same 56 kDa keratin was previously shown by AE1 to be epidermis-specific (Figure 7). Thus the 56 kDa keratin and the 65–67 kDa keratins appeared to be limited to keratinized epidermis.

**AE3 Antibody**

AE3 antibody bound to the 65–67 kDa epidermal keratin (also defined by AE2 antibody), as well as to the 65 kDa cornea-specific keratin (Figure 9; also S. C. G. Tseng, J.-W. Huang and T.-T. Sun, manuscript in preparation). In addition, the antibody stained a diffuse band of approximately 66 kDa in almost all tissues examined (Figure 9). Since a small amount of this 66 kDa protein was also detected in some nonepithelial cells and tissues, including fibroblasts and brain (not shown), this protein did not appear to be a typical keratin and thus was not included in Table 1. This component may be related to a protein reported to share an antigenic determinant with subunits of several types of intermediate filaments (Pruss et al., 1981).

AE3 antibody also recognized a class of 55–58 kDa keratins (referred to as the 58 kDa keratin class) in all stratified squamous epithelia (Figure 9, lanes 1–6), as well as in thymic, bladder and tracheal epithelium (lanes 7–9), but not in the simple epithelia of the gall bladder, diaphragm, liver and stomach (lanes 10–13). The tissue distribution of this 58 kDa keratin class was thus similar to that of the 50 kDa keratin class recognized by AE1 antibody (Figure 7; Table 1). In addition, AE3 detected a 52 kDa keratin and a 46 kDa keratin in a wide variety of epithelium except the epidermis (Figure 9).

**Mixture of Three Antibodies**

Since all major keratins of the epidermis of human, monkey and rabbit were recognized by at least one of the monoclonal antibodies (Figures 7, 8 and 9), a mixture of the three antibodies provided a means to detect in one step all epithelial proteins cross-reacting with epidermal keratins (Figure 10). As expected, the staining pattern represented a sum of the patterns produced by the three separate antibodies. The results showed that keratins of more complex or specialized epithelia are generally larger than the cross-reacting counterparts of simpler epithelia (also see Figures 7 and 9).

The tissue distribution of the seven classes of keratin antigens is summarized in Table 1.

**Discussion**

Keratins have been previously defined by their water-insolubility, their molecular weight range of 40,000–70,000, their ability to assemble into 10 nm tonofilaments, their peptide mapping patterns, their cell and tissue distribution and their reactivities with certain conventional antisera. Monoclonal antibodies specific for keratins provide a refined means for identifying and relating different keratins according to the presence of specific antigenic determinants.

**Keratin Classes**

A large number of keratin species have been described in the literature. However, because of differences in cell type, growth condition and animal species, it has often been difficult to compare results from different laboratories. We have utilized three monoclonal antikeratin antibodies to detect keratins in various monkey epidermis in vivo. These studies revealed a greatly simplified picture. The three antibodies de-

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Figure 7. Immunoblot Analysis of Keratins in Various Monkey and Rabbit Tissues with AE1 Monoclonal Antibody

(a) Monkey and human tissues. All samples were water-insoluble proteins from Cynomolgus monkey unless otherwise specified. (Lane 1) Human abdominal skin; (lane 2) monkey thigh epidermis. Note the staining of 56 kDa keratin in the keratinized epidermis in both species. (Lane 3) Esophageal epithelium; (lane 4) human corneal epithelium; (lane 5) human conjunctiva—this overloaded sample revealed some minor, high molecular weight reaction species presumably representing keratins cross-linked by isopeptide bonds; (lane 6) monkey conjunctiva; (lane 7) thymus; (lane 8) bladder, total proteins; (lane 9) trachea, total—the staining of a high molecular weight component (90 kDa) was sometimes noted; (lane 10) gall bladder, total; (lane 11) diaphragm, peritoneal mesothelium, total; (lane 12) liver, total; (lane 13) stomach, total. Note the staining of a 37 kDa band in lanes 12 and 13.

(b) Rabbit tissues. All samples were water-insoluble proteins unless otherwise specified. (Lane 1) newborn rabbit skin, total proteins; (lane 2) skin, insoluble; (lane 3) cornea; (lane 4) conjunctiva, total; (lane 5) conjunctiva; (lane 6) esophagus; (lane 7) trachea, total; (lane 8) lung, total; (lane 9) stomach, total; (lane 10) small intestine, total; (lane 11) liver, total; (lane 12) kidney, total; (lane 13) pancreas, total.
Figure 8. Detection of Keratins in Monkey and Rabbit Tissues with AE2 Antibody
(a) Monkey tissues; (b) rabbit tissues. Samples are the same as in Figure 7.
Figure 9. Detection of Keratins in Various Monkey Tissues with AE3 Antibody

Samples are the same as in Figure 7a.

Figure 10. Detection of Epidermis-Related Keratins in Various Monkey Tissues with a Mixture of AE1, AE2 and AE3 Antibodies

Samples are the same as in Figure 7a. Lane 14 is skeletal muscle.
defined seven distinct keratin classes (65–67, 58, 56, 52, 50, 46 and 40 kd) whose expression could be correlated with different types of epithelial differentiation (Table 1). These results suggest that specific keratin classes may provide useful molecular markers for the identification of various epithelial cell types both in vivo and in cell culture.

Some keratin classes contained multiple keratin polypeptides. The 56 kd keratin class recognized by AE3 contained at least four components: a 58 kd keratin in all stratified squamous epithelia (Figures 6 and 9), a 55 kd keratin in cornea, a 57 kd keratin in esophagus and a 56 kd basic keratin in cultured human epithelial keratocytes (R. Eichner and T.-T. Sun, manuscript in preparation). Since these keratins shared an antigenic determinant (AE3), and were similar in size (55–58 kd) and charge (basic), they clearly were related and thus were regarded as a class. Similarly, the 50 kd keratin class defined by AE1 antibody contained several acidic components in the molecular weight range of 46–50 kd (Figure 7a).

**Significance of Individual Keratin Classes**

The 65–67 kd and 56 kd keratins were detected only in keratinized epithelia and therefore can be regarded as markers for keratinization. This interpretation is supported by recent immunolocalization data showing that these two keratin classes are present only in terminally differentiating, suprabasally located cells of the epidermis (Sun et al., 1982; Woodcock-Mitchell et al., 1982; see also Viac et al., 1980; Vürich and Sun, 1980). Our findings are also in accordance with previous findings that the 66–67 kd keratins (Doran et al., 1980; Baden et al., 1980; Fuchs and Green, 1981) appear when nonkeratinized, cultured epithelial cells are induced to keratinize. Although the detailed functions of the 65–67 kd and 56 kd keratins are not established, available data suggest that they probably play an important role during keratinization.

The 56 kd and 50 kd classes of keratins were readily detected in all stratified squamous epithelia (as well as in bladder and tracheal epithelia), but not in simple epithelia. These two classes of keratins can therefore be regarded as markers for stratified epithelia (Figures 6, 7 and 9). In view of our recent finding that both 50 kd and 58 kd keratins are present in epidermal basal cells (Woodcock-Mitchell et al., 1982), it seems possible that these two keratins are synthesized in the basal cells of all stratified epithelia.

A 52 kd and a 46 kd keratin were detected by AE3 antibody in almost all epithelia except the epidermis (Figure 9). These two keratins may correspond respectively to a 55 kd keratin (component A) and a 48–49 kd keratin (component D) identified in intestinal epithelial cells and hepatocytes (Franke et al., 1981a and 1981c).

Although the 40 kd keratin and the 46 kd keratin had similar tissue distributions, they were immunologically distinct, for they were recognized by different antibodies (AE1 and AE3 respectively; Figures 7 and 9). As shown recently by Wu and Rheinwald (1981), several human squamous carcinoma cell lines express an increased level of the 40 kd keratin. A similar 40 kd keratin has been found in cultured rabbit corneal epithelial cells (Doran et al., 1980; see also Sun and Green, 1977), in intestinal epithelium (Franke et al., 1981c) and in human amnionic epithelial cells (Keskioja et al., 1981). It is interesting to note, however, that hepatocytes seem to lack the 40 kd keratin (Franke et al., 1981a). Since a small amount of this keratin was detected in the liver (Figure 7), and since AE1 antibody selectively stained bile ducts, it is possible that the 40 kd keratin was present only in the ducts and not in parenchymal cells. A similar situation may exist in pancreas (Figures 2c and 7b, lane 13).

Thus the 40 kd keratin is present in a wide variety of epithelia, except keratinized epidermis (Figure 7) and possibly parenchymal cells of the liver and pancreas. That the process of keratinization may be incompatible with the expression of the 40 kd keratin is suggested not only by our data but also by the recent finding that synthesis of this keratin by cultured keratocytes is inhibited when cells are induced to keratinize (Fuchs and Green, 1981). We also have obtained evidence that this 40 kd keratin is expressed in embryonic epidermis, but disappears shortly before birth as the epidermis becomes fully keratinized (S. C. G. Tseng, J.-W. Huang and T.-T. Sun, manuscript in preparation).

The tissue distribution of the seven classes of keratins is summarized in Table 1. The results indicate that keratinized epidermis expresses 65–67, 58, 56 and 50 kd keratins; nonkeratinized stratified squamous epithelia express 56, 52(?), 50, 46(?) and 40 kd keratins; and simple epithelia express only the 52, 46 and 40 kd keratins. The last finding is in excellent agreement with recent results by Franke and coworkers (1981b and 1981c) demonstrating that intestinal epithelial cells contain three major keratins (55, 48 and 40 kd). Since the keratin pattern revealed by our monoclonal antibodies was similar in rabbit, Cynomolgus monkey, Rhinus monkey (data not shown) and human, the patterns of tissue distribution we observed appear to be relatively conserved during mammalian evolution.

**Keratin Families**

Although the overall relationship among various keratin molecules is not known, and its description will eventually have to be based on sequence comparison, our data revealed an interesting relationship. AF1 and AE3 antibodies recognized two mutually exclusive families of keratins. AE1 antibody recognized the 40, 50 and 56 kd keratin classes, whereas AE3 antibody
recognized the 46, 52, 58 and 65-67 kd keratin classes (Table 1). Among keratins recognized by AE1 antibody, the small 40 kd keratin was present in almost all epithelia; the medium-sized 50 kd keratin class was present only in more complex, stratified epithelia; and the 56 kd keratin, the largest of the three, was present only in keratinized epidermis. A parallel situation exists for keratins recognized by AE3 antibody. The 40 kd and 52 kd keratins were present in almost all epithelia, the medium-sized 58 kd keratin class was present only in stratified epithelia and the 65-67 kd keratins were present only in epidermis.

These results raise the following questions. Is it possible that the two mutually exclusive groups of keratins, as defined by AE1 and AE3 monoclonal antibodies belong to separate gene families? If so, what is the relationship between the keratin families expressed in vivo (as described in this study) and the two gene families recently described in cultured epidermal cells (Fuchs et al., 1981)? Is it possible that genes coding for the smaller keratins in a family are more primitive, giving rise to genes coding for larger keratins by gaining sequences when the epithelia become more specialized during evolution (for example, see Doolittle, 1981)? Finally, what is the functional significance of the requirement for larger keratins by more complex epithelia? Answers to these questions should enhance our understanding of the structural basis and biological significance of keratin heterogeneity.

Experimental Procedures

Monoclonal Antibodies

Monoclonal antibodies (AE1, AE2 and AE3) against human epidermal keratins were prepared by the hybridoma technique (Kohler and Milstein, 1975). A detailed description of the preparation of these antibodies will be presented elsewhere (Woodcock-Mitchell et al., 1982). The monoclonality of each hybridoma cell line was established by three criteria: each line was cloned at least three times; each line produced only one extra light chain and one extra heavy chain when produced by sister clones exhibited identical properties. The supernatants contained total protein extracts.

Gel Electrophoresis and Immunoblot Technique

Proteins were separated by SDS-polyacrylamide gel electrophoresis (12.5% acrylamide) as described by Laemmli (1970). The binding of antikeratin antibodies to keratins separated by SDS-polyacrylamide gel electrophoresis was assayed by the immunoblot technique (Towbin et al., 1979). Proteins from unstained polyacrylamide gels were transferred electrophoretically onto nitrocellulose paper (Millipore) by an E-C blotting apparatus (2.5 hr at 4°C with a power supply setting of 66%). Then stained with the monoclonal antibodies by the peroxidase-antiperoxidase technique ( Sternberger, 1979; Glass et al., 1980; Woodcock-Mitchell et al., 1982).

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