Comparative Studies on Naturally Occurring Antikeratin Antibodies in Human Sera

Keiji Iwatsuki, M.D.,* Jacqueline Viac, Ph.D., Alain Reano, Ph.D., Anna Morera, Ph.D., Marie-Jeanne Staquet, Ph.D., Jean Thivolet, M.D., and Jean-Claude Monier, M.D.

INSERM U.209, Hôpital E. Herriot et Laboratoire d'Immunologie, Faculté Alexis Carrel, Lyon, France

Comparative studies on the specificity of the so-called antiepidermal antibodies (Abs) found in human sera were performed by immunoblotting, enzyme-linked immunosorbent assay (ELISA), and immunoelectron microscopy (IEM). After a screening test by indirect immunofluorescence (IF), sera obtained from patients with various diseases and controls could be classified in 5 different groups according to the IF patterns on the epidermis: sera reactive with: (1) the stratum corneum (SC); (2) the upper layer (U-Cyt); (3) the whole epidermis (G-Cyt); (4) basal cells (B-Cyt); and (5) negative ones. By immunoblotting, all the 23 IF-positive sera were found to bind to one or more keratin bands, and did not show any reactivity with epidermal Nonidet P-40 soluble proteins. SC-Abs were mainly directed against a 67 kD Keratin band, whereas U-Cyt- and G-Cyt-Abs bound to both 58–56 kD and 67–63 kD keratins. B-Cyt-Abs reacted strongly with 63 kD keratins and slightly with a 50 kD band. Antikeratin Abs were detected by immunoblotting even in the IF-negative sera. The ELISA study showed that sera with high IF titers contained high levels of antikeratin Abs. In the IEM study using sera containing U-Cyt- or B-Cyt-Abs, 2 distinct reaction patterns were demonstrated: U-Cyt-Abs stained tonofilaments of suprabasal keratinocytes, while B-Cyt-Abs characteristically reacted with those of basal cells. Moreover, SC-, U-Cyt-, and G-Cyt-Abs were absorbed out by insoluble epidermal proteins, and B-Cyt-Abs were decreased in titer after the absorption test. The present study provides strong evidence that most, though not all, human antiepidermal Abs are directed against different keratin polypeptides, and that antikeratin Abs commonly occur in almost all human sera. *J Invest Dermatol 87:179–184, 1986*

Autoantibodies against cytoplasmic structures of keratinocytes have been commonly detected in human sera [1–3]. They are known to occur in sera of patients with autoimmune diseases [4,5], Waldenström's macroglobulinemia [6], various kinds of skin disorders such as cancers [3,7,8], burns [9], drug eruptions [10], graft-vs-host reactions [11], and even in some sera obtained from normal persons [8]. Previous indirect immunofluorescence (IF) studies [2,3] have demonstrated 3 different types of epidermal cytoplasmic antibodies (Abs): sera reactive with the upper layer of epidermis (U-Cyt), the general epidermis (G-Cyt), and basal cells (B-Cyt). These anticytoplasmic Abs have been considered to be related to some differentiation antigens [12]. In addition, antistratum corneum (SC) Abs have been reported to be found in sera taken from normal individuals as well as patients with skin diseases, and increased in titer under certain skin conditions [13]. Recent studies using an immunoblotting technique [14,15] have been able to demonstrate that U-Cyt- and SC-Abs are directed against keratin intermediate filament proteins, and that they are much more common than previously appreciated.

In the present study, we performed comparative studies to clarify the specificity of human antiepidermal Abs against keratins, using immunoblotting, enzyme-linked immmunosorbent assay (ELISA), and immunoelectron microscopy (IEM).

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*Present address: Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan.

Reprint requests to: Jean Thivolet, M.D., INSERM U.209, Pav. R, Hôpital E. Herriot, 69437 Lyon Cx 03, France.

Abbreviations:

Abs: antibodies
B-Cyt: basal cells
BSA: bovine serum albumin
ELISA: enzyme-linked immunosorbent assay
FITC: fluorescein isothiocyanate
G-Cyt: whole epidermis
IEM: immunoelectron microscopy
IF: immunofluorescence
kD: kilodalton(s)
NP-40: Nonidet P-40
OD: optical density
PAGE: polyacrylamide gel electrophoresis
SC: stratum corneum
SDS: sodium dodecyl sulfate
TBS: Tris-buffered saline
U-Cyt: upper layers (of epidermis)

MATERIALS AND METHODS

Serum Samples Sera reactive with normal epidermis were selected by an indirect IF study for the purpose of screening autoantibodies, using frozen sections of rabbit lip, mouse kidney and liver as substrates (Clinique Dermatologique, Hôpital E. Herriot, Lyon). Some sera were the kind gifts of Dr. Youinou (Centre Hospitalier Brest, France) and of Dr. Yamada (Hamamatsu University, Japan). All test sera were checked again at a dilution of 1:10 on cryostat sections of normal foreskin and rabbit lip. In order to block nonspecific binding of a fluorescein isothiocyanate (FITC)-labeled goat antihuman immunoglobulin serum (Nordic, Netherland) to the epidermis, the sections were treated with an unlabeled goat serum at 1:20, followed by incubation with the

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1:40 diluted FITC conjugate. Sera without any detectable antiepidermal Abs were used as a control group for the following studies.

Extraction of Epidermal Keratins Normal human epidermis was separated from dermis by incubation with 0.5 M EDTA, pH 7.5, at 60°C for 1 min, and keratin proteins were purified and extracted as already described [16]. Briefly, the pellet obtained after several homogenizations of whole epidermal sheets in high-salt buffer was dissolved in 10 mM Tris-HCl, pH 8.0, 0.5% sodium dodecyl sulfate (SDS), and 5% 2-mercaptoethanol, and heated at 60°C for 2 h. The extracted keratin proteins were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) in an 8.5% slab gel [17].

Extraction of Nonidet P-40 (NP-40) Soluble Epidermal Proteins The epidermal sheets were subjected to 2 min of homogenization with a polytron at 4°C in extraction buffer composed of 0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.5% NP-40, 10 mM EDTA, 1 mM phenylmethyl-sulfonyl-fluoride, 5 mM N-ethyl maleimide. The supernatant was analyzed by SDS-PAGE of a 5–15% gradient of polyacrylamide. As shown in the previous report [18], the NP-40 extraction buffer effectively solubilized numerous proteins from epidermis except for keratins.

Immunoblotting Technique Keratins and soluble proteins of the epidermis were separated by SDS-PAGE, followed by transblotting to nitrocellulose paper at 4°C for 3 h [19]. The nitrocellulose paper was divided into 5 mm-wide strips, and incubated with 10 mM Tris-buffered saline pH 7.4 (TBS) containing 5% bovine serum albumin (BSA). Each strip was reacted with 2.5 ml of the variously diluted sera in TBS with 3% BSA at 4°C, overnight. In the screening test by immunoblotting, the test sera were diluted at 1:400. The strips were washed 4 times with TBS containing 0.05% NP-40, and incubated with peroxidase-labeled $F(ab')_2$ fraction of sheep antihuman immunoglobulins (Amersham, U.K.) at a dilution of 1:200 in TBS and 3% BSA, at room temperature for 2 h. After several washings, the peroxidase activity was visualized by incubation with a 0.04% amino-ethyl-carbazole solution.

Enzyme-Linked Immunosorbent Assay Each well of polystyrene microplate (Nunc, Netherland) was coated with 50 μl of keratin extract (10 μg/ml in bicarbonate buffer, pH 9.6), or with 1:5 diluted sheep serum for a control study, at 4°C overnight. The plates were washed 3 times with PBS, and incubated with the test sera or a standard human serum with a threshold value of antikeratin Abs, at 1:10 in PBS containing 0.5% Tween-20 and 10% sheep serum at room temperature for 1 h with agitation. The threshold value of the standard serum corresponds to the 95th percentile established by screening 100 sera from normal subjects. After washing 3 times with PBS-Tween 20, the plates were reacted with 1:400 diluted peroxidase-labeled sheep antihuman IgG (H+L) (Institut Pasteur, Paris) at room temperature for 45 min, followed by washing 4 times with PBS-Tween 20. Peroxidase activity was determined by a colorimetric reaction with Trinder's reagent (25 mM phenol, 2 mM amino-4-phenagine, 0.8 mM H_2O_2 in PBS, pH 7.2) at 37°C for 60 min. Optical density (OD) of each well was read at 492 nm. The absorbance of the keratin-coated wells in the presence of Trinder's reagent was taken as the optical blank. The results were expressed by the following ratio: OD of wells coated with keratins and test sera − those with the sheep serum and test sera / OD of wells coated with keratins and the standard serum − those with the sheep serum and the standard serum.

Immunoelectron Microscopy For IEM observations, 2 different methods were carried out to confirm the specificity of immunolabeling and to clarify the distribution of the reactive keratinocytes in the epidermis. At first, 14-μm nonfixed, frozen sections of normal skin were used as substrates. They were placed on egg albumin-coated glass slides and air-dried. These sections were incubated with 1:5 diluted test sera at room temperature for 2 h. After washing, they were reacted with peroxidase-labeled $F(ab')_2$ fraction of sheep antihuman immunoglobulins (Amersham, U.K., working dilution 1:20) at room temperature for 1 h. Peroxidase staining was achieved by incubation with 0.05 M Tris-HCl buffer pH 7.4, 0.05% 3,3'-diaminobenzidine, and 0.01% H_2O_2. After the specific reaction products were found to be visualized under a light microscope, the sections were fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, followed by embedding with Epon.

Second, immunoperoxidase staining was performed using epidermal cell suspensions. Isolated epidermal cells were obtained from normal human skin by trypsinization, and fixed with 3% paraformaldehyde at 4°C for 30 min. About 5×10^6 isolated epidermal cells were incubated with an undiluted test serum at 4°C overnight, washed in PBS for 8 h, and then reacted with the peroxidase-conjugate at a dilution of 1:10 at 4°C overnight. After washing in PBS for 6 h and successive fixation with 2.5% glutaraldehyde, peroxidase staining was carried out in the same procedures described above. Ultrathin sections were observed under a Philips EM-300 with or without counterstaining.

In each experiment, controls were ascertained by using PBS and IF-negative human sera instead of IF-positive human sera.

Absorption Studies Insoluble epidermal proteins were obtained from the epidermal sheets by the same procedures for purification of keratins described above. Two hundred microliters of each of 1:10 diluted test sera were absorbed with 80 mg wet weight of the insoluble epidermal proteins. After incubation at room temperature for 2 h with subsequent incubation at 4°C for 48 h with agitation, the sera were centrifuged at 17,000 rpm for 10 min. The supernatants were studied for detection of titers of the remaining antiepidermal Abs by indirect IF. A serum containing both bullous pemphigoid Abs and G-Cyt-Abs were used as a control to evaluate the nonspecific protein-to-protein binding in the process of absorption.

RESULTS

Epidermal Fluorescence Patterns According to the epidermal staining patterns, positive sera could be classified in 4 groups as previously described [2,13] (Fig 1); sera reactive with: (1) the stratum corneum without other types of cytoplasmic Abs (SC); (2) the cytoplasm of upper layer of the epidermis (U-Cyt); (3) the general epidermis (G-Cyt); and (4) the cytoplasm of basal cells (B-Cyt). The sera in the latter 3 groups were frequently accompanied by SC-Abs.

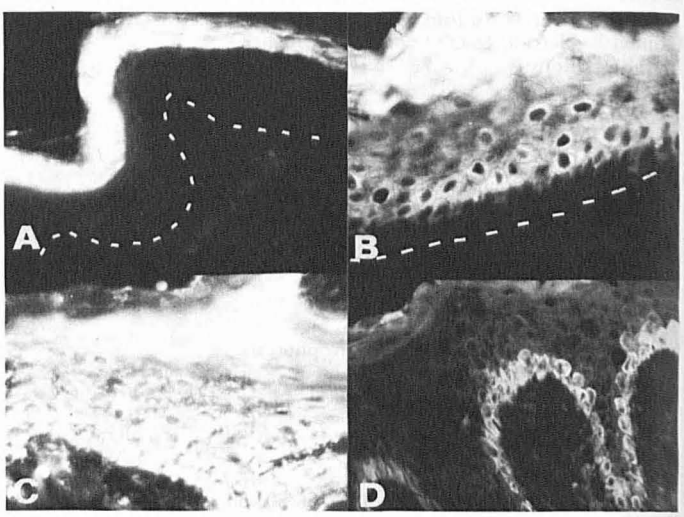

Figure 1. IF reactivity pattern of 4 different types of antiepidermal cytoplasm Abs. (*A*) SC-, (*B*) U-Cyt-, (*C*) G-Cyt-, and (*D*) B-Cyt-Abs.

Although SC and U-Cyt-Abs were quite common among the sera tested, the exact incidence of each type of antiepidermal Abs was not able to be determined, because the sera were obtained from patients with special disease conditions in which antiepidermal Abs are thought to occur more frequently.

Results of Immunoblotting Keratin polypeptides transferred onto nitrocellulose sheets revealed 5 major bands at 67 kD, 63 kD, 58 kD, 56 kD, and 50 kD (Fig 2, lanes 1 and 10). All the 23 IF-positive sera reacted with one or more keratin protein bands. As shown in Table I and Fig 2, the test sera reacted with keratin protein bands in a different manner, which made a characteristic reaction pattern of each group, classified by IF staining. Thus, all SC-Abs reacted mainly with a 67 kD keratin band although some of them stained 63 kD, 58 kD, and 56 kD bands faintly (Fig 2, lanes 2 and 3). In contrast, some U-Cyt-Abs showed a strong reactivity against both 67–63 kD and 58–56 kD keratins (Fig 2, lane 4). In this group, the reaction with 58–56 kD keratin bands was more frequent and more intense than in other groups (Fig 2, lanes 4 and 5). G-Cyt-Abs showed positive reactions at 67–63 kD and 58–56 kD similar to those of U-Cyt-Abs (Fig 2, lane 6). On the other hand, 2 B-Cyt-positive sera revealed a characteristic staining picture; they reacted strongly with a 63 kD band and one of them stained a 50 kD band weakly (Fig 2, lanes 8 and 9). A faint reactive band at 67 kD occasionally appeared which may be due to slight reactivity of the serum with the SC (Fig 2, lane 8).

The results of comparative titration of antikeratin Abs demonstrated that our immunoblot system is more sensitive than indirect IF study, and that IF-negative sera also possess antiepidermal Abs in low titers. In fact, in the screening test by immunoblotting at a dilution of 1:400, all the 10 IF-negative sera showed a reactivity with various intensity against 67–63 kD bands often associated with positive reactions at 58–56 kD (Table I). Six samples of human sera (4 IF-positive and 2 IF-negative) tested in immunoblot at various dilutions (1:400 to 1:6400) showed a decrease in the band reactivities. All samples were negative at 1:6400. The IF-negative sera still reacted at 1:400 dilution whereas the IF-postive sera remained positive at 1:3200 or 1:6400 dilution.

The reactivity of antiepidermal Abs against epidermal NP-40 soluble proteins was checked by immunoblotting using 5 selected sera containing SC-(no.1) U-Cyt-(nos. 8,9), G-Cyt-(no. 20), or B-Cyt-(no. 22)-Abs. None of them showed any reactivity with soluble proteins ranging from 20 kD to 250 kD at dilutions of 1:200 and 1:400.

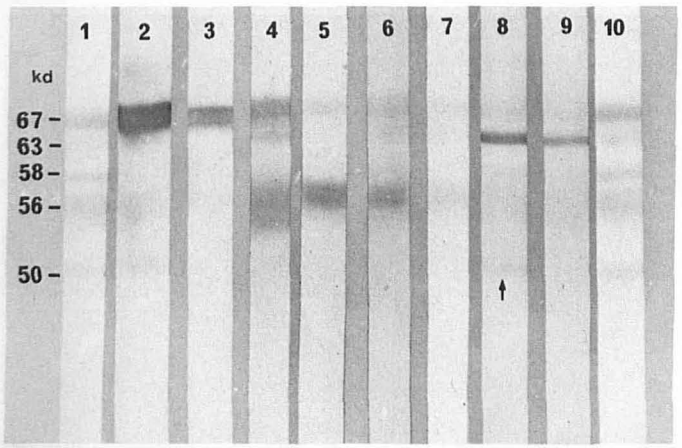

Figure 2. Immunoblot reactions against keratins. *Lanes 1 & 10*: Keratins separated by SDS-PAGE and stained with fast green on nitrocellulose paper; *lanes 2 & 3*: SC-Abs; *lanes 4 & 5*: U-Cyt-Abs; *lane 6*: G-Cyt-Abs; *lane 7*: an IF-negative serum; *lanes 8 & 9*: B-Cyt-Abs. The *arrow* indicates a faint reactivity with a 50 kD band.

Table I. Results of Immunoblot and ELISA on Individual Samples Tested

Sample No.	IF Pattern (titer)	Reactive Keratin Proteins at 1:400 Dilution (kD)					ELISA OD Ratio[a]
		67	63	58	56	50	
1	SC (1:80)	++	+	±	±	−	0.30
2	SC (1:10)	++	±	−	+	−	0.44
3	SC (1:40)	++	±	+	+	±	1.89[b]
4	SC (1:40)	++	±	±	±	−	1.27[b]
5	U-Cyt (1:40)	+	+	+	+	−	0.72
6	U-Cyt (1:40)	++	+	+	+	−	1.08[b]
7	U-Cyt (1:40)	++	+	+	+	−	0.84
8	U-Cyt (1:160)	++	+	++	++	−	2.15[b]
9	U-Cyt (1:80)	++	+	+	+	−	2.50[b]
10	U-Cyt (1:10)	++	±	+	+	−	0.29
11	U-Cyt (1:40)	++	±	±	±	−	1.40[b]
12	U-Cyt (1:80)	++	±	++	++	−	1.51[b]
13	U-Cyt (1:40)	++	±	++	++	−	2.48[b]
14	U-Cyt (1:80)	+	++	++	++	−	2.08[b]
15	U-Cyt (1:80)	+	+	±	+	−	1.38[b]
16	U-Cyt (1:10)	±	−	−	±	−	0.25
17	U-Cyt (1:40)	++	−	−	++	−	0.53
18	U-Cyt (1:40)	±	±	−	+	−	3.04[b]
19	U-Cyt (1:40)	++	±	+	+	−	3.61[b]
20	G-Cyt (1:10)	+	+	+	+	−	0.77
21	G-Cyt (1:40)	+	−	±	+	−	1.47[b]
22	B-Cyt (1:1280)	±	++	−	−	+	1.64[b]
23	B-Cyt (1:20)	−	++	−	−	−	0.72
24	Neg	++	±	±	+	−	0.35
25	Neg	++	±	−	±	−	0.99
26	Neg	++	−	±	±	−	0.40
27	Neg	±	±	+	+	−	1.12[b]
28	Neg	+	−	−	+	−	0.70
29	Neg	+	−	−	±	−	0.76
30	Neg	±	−	−	−	−	0.47
31	Neg	+	−	−	+	−	0.94
32	Neg	±	±	−	−	−	ND
33	Neg	±	−	±	−	−	ND
34	Neg				ND		0.55
35	Neg				ND		0.93
36	Neg				ND		0.41
37	Neg				ND		0.80
38	Neg				ND		0.83
39	Neg				ND		0.30
40	Neg				ND		0.57

[a]See text.
[b]Indicates sera beyond threshold value (95th percentile).
Key: ND = not done.
Neg = negative at a dilution of 1:10.
++ = strong, + = moderate, ± = weak intensity.

Results of ELISA As shown in Fig 3, the sera showing positive fluorescence for the epidermal cytoplasm contained higher levels of antikeratin Abs by the ELISA study. The average titer of antikeratin Abs in this group was significantly increased (1.41 ± 0.93, $p < 0.01$) as compared with that of the IF-negative group (0.67 ± 0.26). However, each titer of cytoplasmic Abs determined by the IF study did not always correlate to the results of ELISA (Table I). For most samples, there are no linear relationships between the IF titers and the ELISA OD ratios.

The numbers of sera showing high titers of antikeratin Abs beyond the threshold value of the standard serum were 2 of 4 SC-Abs, 10 of 15 U-Cyt-Abs, 1 of 2 G-Cyt-Abs, 1 of 2 B-Cyt-Abs, and 1 of 15 IF-negative sera (Table II).

IEM Study Sera containing U-Cyt (no. 9) or B-Cyt-Abs (no. 22) were examined by the IEM technique. When normal skin specimens were utilized as a substrate, U-Cyt-Abs reacted with only the cytoplasm of suprabasal cells including corneocytes. In contrast, B-Cyt-Abs stained the cytoplasm of basal cells and some corneocytes but not of spinous cells. The 2 distinct staining pat-

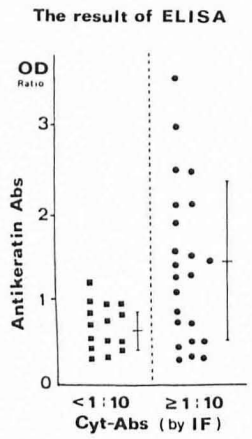

Figure 3. Results of ELISA. Cyt-Abs-positive sera contain higher levels of antikeratin Abs (1.41 ± 0.93, $p < 0.01$) as compared with negative ones at a dilution of 1:10 (0.67 ± 0.26).

terns provided the evidence for the specificity of the reaction produced by each serum. At higher magnifications, the reaction products were found to coincide with tonofilaments in the cytoplasm of suprabasal cells in the U-Cyt-stained sections, and with fine tonofibrils in the basal cells in the B-Cyt-reacted sections (Fig 4*C*).

In cell suspensions stained with U-Cyt-Abs, the positive keratinocytes were clearly distinguished from the negative ones. The latter had numerous melanosomes, fine tonofilaments, and desmosomal structures suggestive of basal cells (Fig 4*A*). Corneocytes and cells in the granular layer were also stained with the U-Cyt-Abs. When cell suspensions were stained with B-Cyt-Abs, tonofilaments of the basal cell-like cells were characteristically stained, which showed apparent contrast to the staining picture of U-Cyt-Abs (Fig 4*B*). However, some corneocytes and cells in the granular layer were occasionally positive for the serum.

Results of Absorption Test Antiepidermal Abs against SC (no.1), U-Cyt (no. 9), and G-Cyt (nos. 20, 21) (IF titer see Table I) were absorbed out by insoluble proteins obtained from the epidermis (IF titer < 10). The titer of B-Cyt-Abs (no. 22), which showed an initial titer of 1:1280, was decreased to 1:20. In the serum containing both pemphigoid Abs (1:1280) and G-Cyt-Abs (no. 21) (1:40), G-Cyt-Abs were removed after the absorption test (IF titer < 10), but on the other hand pemphigoid Abs showed no change in the titer (Fig 5).

DISCUSSION

Antiepidermal Abs are known to occur frequently in human sera and to represent at least 4 different epidermal staining patterns by indirect IF: SC-, U-Cyt-, G-Cyt-, and B-Cyt-staining [2,8,13]. The previous IF studies using polyclonal or monoclonal Abs raised by immunization with keratins showed epidermal cytoplasmic fluorescence similar to those obtained by human antiepidermal Cyt-Abs [20–22]. Moreover, Hintner et al [14,15] have demonstrated by immunoblotting that both SC- and U-Cyt-Ab are directed against keratin intermediate filament proteins. These findings have suggested the possibility that keratin proteins bear antigenic moieties against some kinds of antiepidermal Abs detected in human sera.

As shown in the present immunoblotting study, not only SC- and U-Cyt-Abs but also G-Cyt- and B-Cyt-Abs were reactive with one or more keratin protein bands. In addition to the results of immunoblotting, the ELISA study provided quantitative correlations between the amount of antikeratin Abs and the titers of antiepidermal Abs determined by the IF study; in some cases, sera containing high IF titers of antiepidermal Abs represented the highest levels of antikeratin Abs by ELISA. However, there is no linear relationship between the results of the 2 tests (see Table I). Furthermore, the IEM observations demonstrated that the U-Cyt- and B-Cyt-Abs reacted exclusively with tonofilaments of suprabasal cells and basal cells, respectively. These 2 distinct staining patterns clearly showed the specific binding sites of each antiepidermal Abs on the epidermis. In the absorption test, SC-, U-Cyt-, and G-Cyt-Abs were absorbed out by insoluble epidermal proteins, and B-Cyt-Abs were decreased in titer form 1:1280 to 1:20.

Based on the results obtained from comparative studies by different procedures (Table II), it seems most likely that antiepidermal Abs in human sera are directed against keratin proteins. However, the present study does not deny the possibility that cytoplasmic components other than keratins bear antigenic moieties for certain types of Cyt-Abs. At least one human serum has been shown to be reactive by IEM with a nonkeratin substance in the peripheral parts of keratinocyte cytoplasm [12].

As regards the correlation between IF patterns of Cyt-Abs and reactive keratins, previous IF studies using polyclonal Abs against high-molecular-weight keratin polypeptides showed the U-Cyt staining of the epidermis [20]. More recently, Sun et al [21] demonstrated that monoclonal antikeratin Abs which showed selective staining of basal cells reacted with low-molecular-weight keratins, whereas Abs that stained the suprabasal or entire epidermis bound to the high-molecular-weight keratins. Our immunoblot study also showed some correlations between the IF

Table II. Summary of Comparative Studies on Antiepidermal Cytoplasmic Antibodies

IF Pattern	Immunoblot		ELISA		IEM	Absorption by Insoluble Epidermal Proteins
	No. of Positive Sera	Mainly Reactive Keratins (kD)	No. of Sera Beyond the Threshold[a]	(mean titer of antikeratin Abs)	Sites of Reaction	
SC	4/4	(67[b], 63, 58, 56)	2/4	(0.98 ± 0.75)	ND[c]	+
U-Cyt	15/15	(67[b], 63, 58[b], 56[b])	10/15	(1.60 ± 1.02)[d]	Tonofilaments of suprabasal cells	+
G-Cyt	2/2	(67, 63, 58, 56)	1/2	(1.1)	ND	+
B-Cyt	2/2	(63[b], 50)	1/2	(1.2)	Tonifilaments of basal cells and some corneocytes	+
Negative	10/10	(67[b], 58, 56)	1/15	(0.67 ± 0.26)	ND	

[a]The threshold value of the standard serum corresponds to the 95th percentile established by screening 100 sera from normal subjects.
[b]Indicates strong reactive bands of keratin polypeptides.
[c]ND = not done.
[d]$p < 0.01$ (vs a negative group).

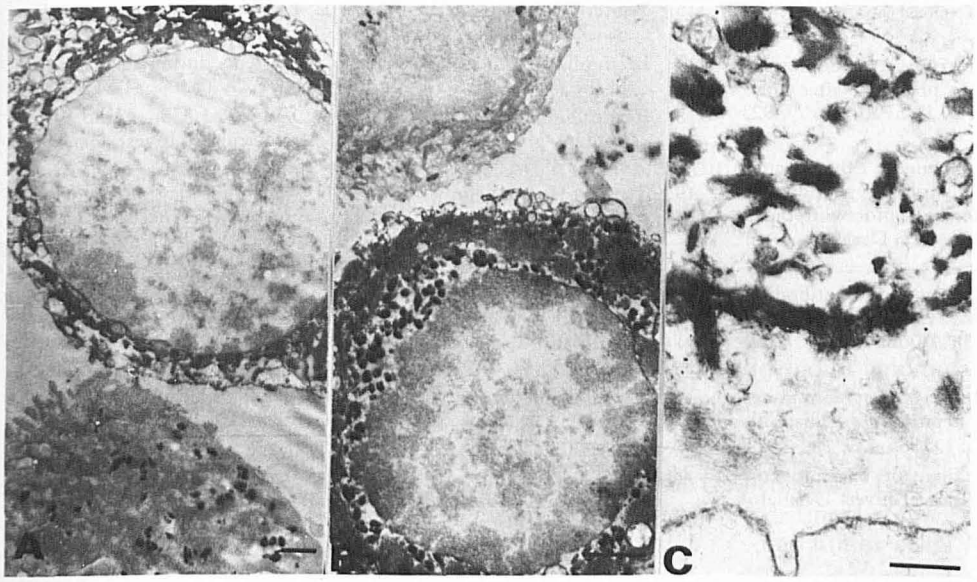

Figure 4. IEM Studies. (*A*) Keratinocyte reactive with U-Cyt-Abs is distinct from a negative basaloid cell including fine tonofilaments and numerous melanosomes in cytoplasm. (*B*) Basaloid cell is characteristically stained with B-Cyt-Abs. (*C*) U-Cyt-Abs react with tonofilaments of suprabasal keratinocytes. *Bars* = 1 μm (*A & B*), 0.5 μm (*C*).

patterns of antiepidermal Abs and the reactive keratin bands. Thus, the sera containing only SC-Abs bound mainly to a 67 kD band, and U-Cyt-Abs showed a strong reactivity with both 67–63 kD and 58–56 kD keratins. However, attention should be paid to the fact that despite showing the same U-Cyt staining by the IF study, the sera did not always react with the same keratin protein bands. This finding may suggest the heterogeneity of this group of Cyt-Abs. On the other hand, sera containing B-Cyt-Abs, which were a rare type of Abs, reacted strongly with a 63 kD band and slightly with a 50 kD band. It is well known that 63 kD is not expressed in basal cells and the discrepancy between IF and immunoblotting remains unclear in this case. However IEM clearly demonstrated a reactivity with tonofibrils of basal cells. There was only a faint reaction product at the 67 kD band which was more frequently reactive with other types of antiepidermal Abs. Although this reaction pattern seems characteristic of B-Cyt-Abs, the number of tested sera is limited so that for the present we can hardly draw a definite conclusion. These findings, in addition to the previous reports [20–22], may suggest that naturally occurring antiepidermal Abs also recognize different antigen moieties on keratin polypeptides expressed in the process of epidermal differentiation.

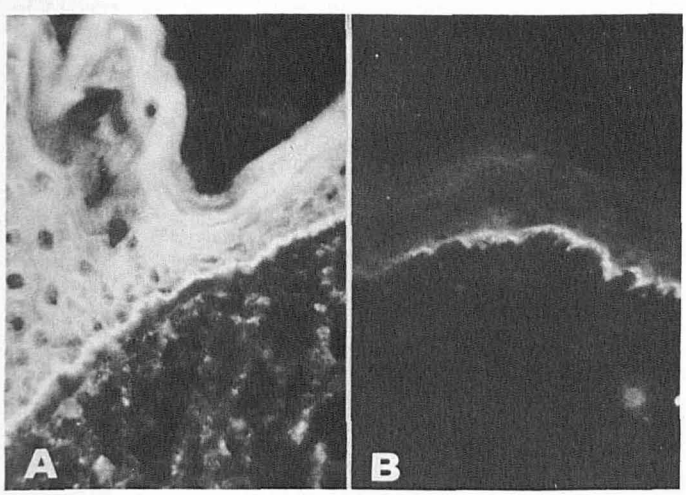

Figure 5. IF results of the absorption studies. Before (*A*) and after (*B*) absorption test; G-Cyt-Abs were absorbed out by insoluble epidermal proteins leaving behind basement membrane zone Abs.

Low titers of antikeratin Abs were detected in almost all the IF-negative sera by immunoblotting and showed similar reaction patterns to those of SC- or U-Cyt-Abs. This result may be explained by the fact that immunoblotting is 10–160 times more sensitive that indirect IF technique [14].

In conclusion, the present study demonstrated that keratin proteins bear antigenic moieties for antiepidermal Abs and that antikeratin Abs occur frequently in human sera. However, it remains obscure whether keratins or denatured keratin proteins are actual immunogens for production of autoantibodies in humans, or antikeratin Abs are produced by exogenous cross-reacting antigens with subsequent destruction of the tolerance against "self." Furthermore, the pathogenic role of antiepidermal Abs, especially the effect on necrotic epithelial cells with naked keratins, is still unanswered.

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