The 105-kDa Basement Membrane Autoantigen p105 Is *N*-Terminally Homologous to a Tumor-Associated Antigen

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Certain constitutive skin basement membrane components, such as bullous pemphigoid antigens and epidermolysis bullosa acquisita antigen, were discovered because they were targeted by an autoimmune reaction. We aimed to purify and characterize a 105-kDa skin basement membrane protein termed p105 recognized by autoantibodies (anti-p105) from patients with a unique immune-mediated subepidermal blistering skin disease. A simian virus 40-transformed human fibroblast cell line that synthesizes and secretes p105 was utilized as the protein source. p105 was partially purified by salt-gradient fractionation of serum-free conditioned medium through a Mono Q anion-exchange column and by examining each fraction with protein staining and immunoblotting against anti-p105. p105 was isolated from polyacrylamide gel electrophoresis gels, blotted onto polyvinylidene difluoride membrane, and subjected to protein microsequencing. The 20 microsequenced *N*-terminal amino acids exhibited no homology to known basement membrane proteins but exhibited a 70% homology to a 90-kDa tumor-associated antigen. Antibodies raised against a peptide generated from these amino acid sequences reacted to a 105-kDa western-blotted keratinocyte and fibroblast protein and a basement membrane component. p105 resisted digestion by glycosidases chondroitinase ABC, neuraminidase, and N-glycosidase F but was cleaved by protease V8 to antigenic fragments of 22 kDa and 14 kDa. The synthesis of p105 was inhibited by cycloheximide. We conclude that p105 is a unique basement membrane component produced by both keratinocytes and fibroblasts. ***Key words: amino acid sequence/chromatography/ion exchange/autoantibodies/autoimmune diseases/ blister. J Invest Dermatol 107:209–214, 1996***

Some constitutive components of the basement membrane zone (BMZ) between the epidermis and dermis were identified because they were targets of primary immunobullous diseases of the skin. The bullous pemphigoid antigens (Stanley *et al*, 1981; Labib *et al*, 1986; Mueller *et al*, 1989), and the epidermolysis bullosa acquisita antigen (Woodley *et al*, 1984) are such examples. The protein and molecular structures of these components were subsequently delineated using the patients' autoantibodies as probes (Stanley *et al*, 1988; Diaz *et al*, 1990; Parente *et al*, 1991). As a result of studies on human diseases, the normal structure and function of human skin BMZ is now better understood (Uitto and Christiano, 1992). We previously identified a 105-kDa autoantigen (p105) detected by autoantibodies (anti-p105) in two patients who had a new form of subepidermal blistering skin disease (Chan *et al*, 1993; Chan and Cooper, 1994; Cotell *et al*, 1994). This newly identified disease, termed "anti-p105 pemphigoid," is distinct from other known blistering skin diseases by clinical, histopathologic, and immunopathologic criteria. p105 is localized within the dermal-epidermal junction of normal human skin and within the epithelial BMZ of normal human mucosae (Chan *et al*, 1993). Interestingly, the *in vivo* expression of p105 is defective or absent (Chan *et al*, 1995a) in the skin of patients with generalized dystrophic epidermolysis bullosa, a hereditary blistering skin disease characterized by defective type VII collagen (Leigh *et al*, 1988; Ryynanen *et al*, 1992). By direct and indirect immunoelectron microscopy, p105 is localized to the lower lamina lucida area of the skin BMZ (9), similar to laminin-5 (also known as epiligrin/kalinin/BM600/nicein), another BMZ component implicated in a group of patients with a type of cicatricial pemphigoid (Domloge-Hultsch *et al*, 1992). Comparative biochemical data revealed that p105 is an acidic protein synthesized and secreted by epidermal keratinocytes and dermal fibroblasts (Chan *et al*, 1995b) and is distinct from the 105-kDa truncated laminin-5 γ2 chain (Carter *et al*, 1991; Rousselle *et al*, 1991; Burgeson *et al*, 1994). In this study, we further characterized this BMZ component p105.

MATERIALS AND METHODS

Cell Cultures and Protein Preparation A simian virus 40-transformed human fibroblast cell line HSF4-T12 was a generous gift from Charles Goolsby (Goolsby *et al*, 1991). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO-BRL) and 1% penicillin/streptomycin. At early confluency, the cell layers were washed three times with phosphate-buffered saline and incubated with serum-free DMEM

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Abbreviations: BMZ, basement membrane zone; TAA, tumor-associated antigen.

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for the next 12–24 h. The serum-free conditioned medium was then collected. After cellular debris was removed by centrifugation (1200 rpm, 4°C, 10 min), the medium was used as the source for protein purification after the concentrations were measured by a Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Keratinocyte and fibroblast cytosol proteins were prepared as previously described (Chan *et al*, 1993).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting SDS-PAGE was performed using 6% SDS-PAGE slab gels (Novex, San Diego, CA) according to an established method (Laemmli, 1970). The proteins loaded onto the gel slots were vertically separated by a constant voltage operation (125 V) under reducing conditions for 2 h. For immunoblotting, the proteins separated by SDS-PAGE were horizontally transferred to nitrocellulose membranes (Bio-Rad) at constant voltage (33 V) for 2 h. The transfer efficiency was examined by a reversible protein stain Ponceau S (0.1% in 5% acetic acid; Sigma Chemical Co., St. Louis, MO). The membranes were then washed with water, blocked with 5% nonfat powdered milk, and then reacted with primary antibodies (human or rabbit antiserum) diluted in Tris(hydroxymethyl)-aminomethane (Tris)-buffered saline plus 1% bovine serum albumin (Sigma) and 0.05% Tween-20 (Sigma) at 4°C overnight. The membranes were then incubated with peroxidase-conjugated, goat anti-human IgG (1:5000, γ-chain specific, Kirkeggard & Perry, Gaithersburg, MD) or anti-rabbit IgG (1:500, Cappel Laboratories, Inc., Westchester, PA) at room temperature for 1 h. The immune reactions were subsequently visualized with a standardized peroxidase substrate solution, 4-chloro-1-naphthol (Kirkeggard & Perry).

Mono Q Anion-Exchange Chromatography The entire chromatographic procedure was carried out at room temperature. Q-Sepharose FF was purchased from Pharmacia Biotech, Inc. (Piscataway, NJ). The Sepharose beads (2 ml) were loaded onto a plastic chromatography column (Bio-Rad) in the presence of an equilibration buffer (25 mM Tris-HCl, pH 7.5) (Hammerberg *et al*, 1992). After the column was washed thoroughly with 15 ml equilibration buffer, the serum-free T12 conditioned medium was loaded to the column and passed through by gravity. A total of 10 mg protein was passed through the column. After the unbound proteins were washed off the column with 5 ml equilibration buffer, the bound proteins were eluted from the column by a stepped NaCl gradient in equilibration buffer (0.1–1.0 M, 3 ml per fraction, each succeeding fraction with 0.1 M increment of NaCl concentration). Forty microliters of each of the eluted protein fractions were electrophoresed by a 6% reducing SDS-PAGE and examined by Coomassie Brilliant Blue staining. Similarly, the electrophoretically separated proteins were transferred to nitrocellulose membrane and immunoblotted against anti-p105 antiserum as described in the section above.

Cycloheximide Inhibition At subconfluency, T12 fibroblast monolayers grown in DMEM, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, were treated with cyclohexamide (Sigma) at a final concentration of 1 μM dissolved in dimethylsulfoxide (final dilution 1:100,000). A control sample was treated with dimethylsulfoxide alone at the same final dilution. Twenty-four hours later, the serum-containing medium was removed. After washing with phosphate-buffered saline three times, the cells were placed on serum-free DMEM supplemented with 1% penicillin/streptomycin, plus 1 μM cycloheximide (or dimethylsulfoxide control). Twenty-four hours later the cells were examined for viability, and the serum-free medium was collected and concentrated by a 50-kDa cutoff Centricon concentrator (W.R. Grace, Beverly, MA). The protein concentration was measured by a Bradford assay (Bio-Rad). Equal amount of total proteins (5.5 μg) of the concentrated medium from cycloheximide-treated and control samples were subjected to a 6% reducing SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted against anti-p105 antiserum as described above.

Biochemical Characterization

Proteolytic Enzymes NaCl (0.4 M)-eluted T12 medium sample was mixed with Staphylococcal protease V8 (Boehringer Mannheim, Indianapolis, IN) in 20:1 ratio (sample-enzyme, wt/wt) and incubated at room temperature for 1 h. The resulting mixture was electrophoresed by a 4–20% gradient gel (Bio-Rad) under reducing conditions, transferred to nitrocellulose membrane, and immunoblotted with the anti-p105 antiserum as described above. Control samples included 0.4 M NaCl-eluted T12 medium in the absence of protease V8, and protease V8 alone.

Endo- and Exoglycosidases NaCl (0.4 M)-eluted T12 medium sample was incubated with chondroitinase ABC (Boehringer Mannheim, 0.1 U per 110-μg sample), neuraminidase (Boehringer Mannheim, 0.25 U per 110-μg sample), and N-glycosidase F (Boehringer Mannheim, 1 U per 110-μg sample) at 37°C overnight. The resulting mixture was electrophoresed by a 6% SDS-PAGE under reducing conditions, transferred to nitrocellulose membrane, and immunoblotted with the anti-p105 antiserum as described above. Control samples included a purified glycoprotein, laminin-1 (Sigma), which was treated with the same enzyme (units) per protein (micrograms) ratio.

Protein Microsequencing The 0.4 M NaCl-eluted T12 medium fraction was concentrated with a 50-kDa cut-off Centricon concentrator (W.R. Grace & Co., Beverly, MA), electrophoresed by a 6% reducing SDS-PAGE, and visualized by Coomassie Brilliant Blue. When 15 μg, 30 μg, or 45 μg proteins were loaded to each lane of the slabgel, five well-separated protein bands between 97 kDa and 116 kDa were identified **(Fig 2*a*)**, cut out from three lanes of a slabgel (each lane loaded with 30 μg protein), and eluted with 100 μl elution buffer (5 mM Tris, pH 6.8, 1% SDS, 10% glycerol) by placing the samples on a microcentrifuge tube immobilized on a rapidly rotating shaker at room temperature for 48 h. Thirty microliters of each of the five eluted protein bands were electrophoresed with a 6% reducing SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted against the anti-p105 antiserum as described above in order to determine which of the isolated protein bands is reacted by the anti-p105 antiserum. After the above verification, the concentrated 0.4 M NaCl-eluted medium fraction (250 μg of protein per lane) was electrophoresed by a 6% reducing SDS-PAGE, transblotted onto a polyvinylidene difluoride membrane (Problott, Applied Biosystems, Foster City, CA) at 33 V, 4°C, 3 h, and stained with acetic acid-free Coomassie Brilliant Blue solution (0.25% in 40% methanol) for 1.5 min. The membrane was subsequently destained with 40% methanol and 10% acetic acid for 45 min and rinsed thoroughly with double distilled water (five times, each 3 min). The 105-kDa protein band verified by the above methods was cut out from the membrane and air-dried for 15 min. The dried membrane was sent to the Northwestern University Biotechnology Core Facility for protein microsequencing at the *N* terminus using Edman degradation method on a protein microsequencer (model 477A; Applied Biosystems).

Anti-Peptide Antibody Production In order to verify that the *N*-terminal amino acid sequence determined by microsequencing technique encodes p105, we generated a multiple antigen peptide (Posnett *et al*, 1988; Tam, 1988) from the first 13 of the 20–amino acid sequence by an Fmoc chemical method on a solid-phase peptide synthesis support (Quality Controlled Biochemicals, Hopkinton, MA). The synthetic peptide was purified on a Vydac HPLC column to greater than 90% purity, and the sequence was verified by a Vestec Electrospray Mass Spectrometer (Vestec Corp., Houston, TX). After a pre-immune serum was taken, the peptide, suspended in phosphate-buffered saline at 1 mg per ml, was mixed with an equal volume of complete Freund's adjuvant, emulsified, and injected in two rabbits (200 μg/rabbit) at six to eight subcutaneous sites. The subsequent injections of peptide (150 μg per rabbit) mixed with an equal volume of incomplete Freund's adjuvant occurred at days 35, 55, and 69. At day 85, the rabbits were bled (Quality Controlled Biochemicals), and the antiserum and pre-immune serum were tested for reaction against p105 by immunoblotting against keratinocyte and T12 fibroblast cytosol proteins and by immunofluorescence microscopy.

Indirect Immunofluorescence Microscopy Indirect immunofluorescence microscopy was performed as previously described (Chan *et al*, 1993). Salt-split normal human skin substrates were prepared as previously described (Woodley *et al*, 1983). Cryosections (6–μm-thick) of salt-split skin and intact normal human skin and sections of monkey esophagus epithelium (5–μm-thick) (Immco, Buffalo, NY) were used as basement membrane substrates. The substrates were first incubated with rabbit serum immunized against the multiple antigen peptide or pre-immune serum from the same rabbit, diluted in Tris-buffered saline plus 1% bovine serum albumin, with the addition of either 0.05% Tween-20, 0.3% Nonidet P-40 (Sigma), or 0.5% Triton X100 (Sigma) at room temperature for 30 min, followed by incubation with fluorescein-conjugated goat anti-rabbit IgG (Cappel) for another 30 min. The tissues were then mounted with a glycerol-phosphate-buffered saline medium (1:1, vol/vol), and viewed with a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with epi-illumination.

RESULTS

Mono Q Anion-Exchange Chromatography When serum-free T12 medium fractionated by a stepped sodium chloride gradient was examined by immunoblotting against the anti-p105 antiserum, a 105-kDa immunoreactive protein band was visualized in 0.3, 0.4, and 0.5 M NaCl-eluted fractions. The strongest band was present in the 0.4 M NaCl-eluted fraction **(Fig 1)**.

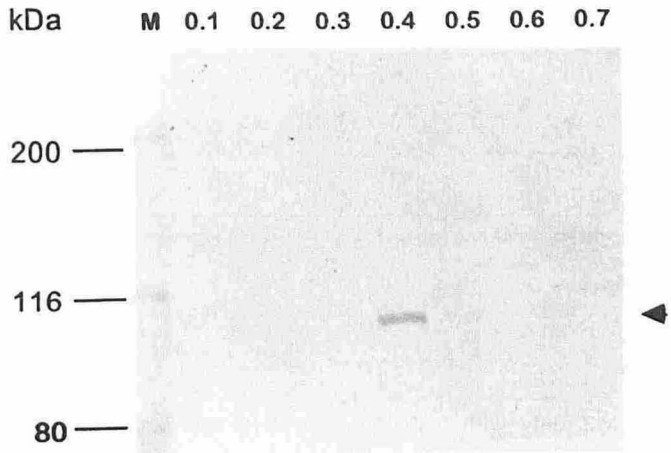

Figure 1. P105 is partially purified by anion-exchange chromatography. Serum-free T12 fibroblast conditioned medium was passed through a Mono Q anion-exchange choromatography column, and the bound proteins were eluted with a stepped increase of NaCl concentration. The protein fractions were separated by a 6% reducing SDS-PAGE, transferred to nitrocellulose membrane, and immunoreacted against the anti-p105 antiserum, followed by visualization with peroxidase-labeled goat anti-human IgG. M = molecular weight standards; 0.1–0.7 = 0.1 M-0.7 M NaCl-eluted fractions; ◀ = p105

Microsequencing of the *N*-Terminal Amino Acids In order to determine the true identity of p105, we used the partially purified 0.4 M NaCl-eluted protein fraction **(Fig 1)** to obtain the amino acid sequence at the *N* terminus. After the 105-kDa protein was confirmed to be the single protein band reacted to the anti-p105 antiserum **(Fig 2)**, the 105-kDa protein blotted onto a polyvinylidene difluoride membrane was isolated for microsequencing. From two independently submitted samples, a stretch of 20 amino acids at the *N* terminus was determined: *N* terminus-S-N-D-G-D-M-R-M-A-D-G-E-D-T-N-Q-G-H-V-E **(Table I)**. By comparing this sequence with other known sequences in the Genbank data base, we found no homology to other BMZ proteins. A 70% homology was found between this sequence and the primary amino acid sequence of a 90-kDa tumor-associated antigen (TAA) (Linsley *et al*, 1986; Rosenberg *et al*, 1991; Iacobelli *et al*, 1993) as depicted in **Table I**.

Biochemical Characterization of p105 In order to delineate the possible glycosylation of p105, to determine whether it may be distinct from other known BMZ components, we subjected the 0.4 M NaCl-eluted T12 medium fraction to endo- and exoglycosidase and proteolytic enzyme digestion. When the protein was subjected to chondroitinase ABC, neuraminidase, or *N*-glycosidase F digestion, the immunoreactive band was unchanged in size, while the control glycoprotein laminin-1 was reduced in size by these glycosidases **(Fig 3)**, suggesting that p105 does not contain significant amounts of carbohydrate. To further delineate the biochemical nature of p105, we subjected the T12 medium fraction to enzymatic digestion by V8 protease (endoproteinase Glu-C). When the 0.4 M NaCl-eluted T12 medium fraction was subjected to V8 protease digestion, p105 was cleaved, producing two immunoreactive fragments of 14 and 22 kDa in size **(Fig 4)**, indicating that p105 contains peptide and ester bonds at the carboxylic side of Glu or at both Glu and Asp.

Metabolic Inhibition of p105 In order to delineate the metabolic pathway of p105 synthesis, cycloheximide was added to T12 fibroblast culture. After 48 h of cycloheximide incubation, the immunoreactive p105 was totally absent from the culture medium,

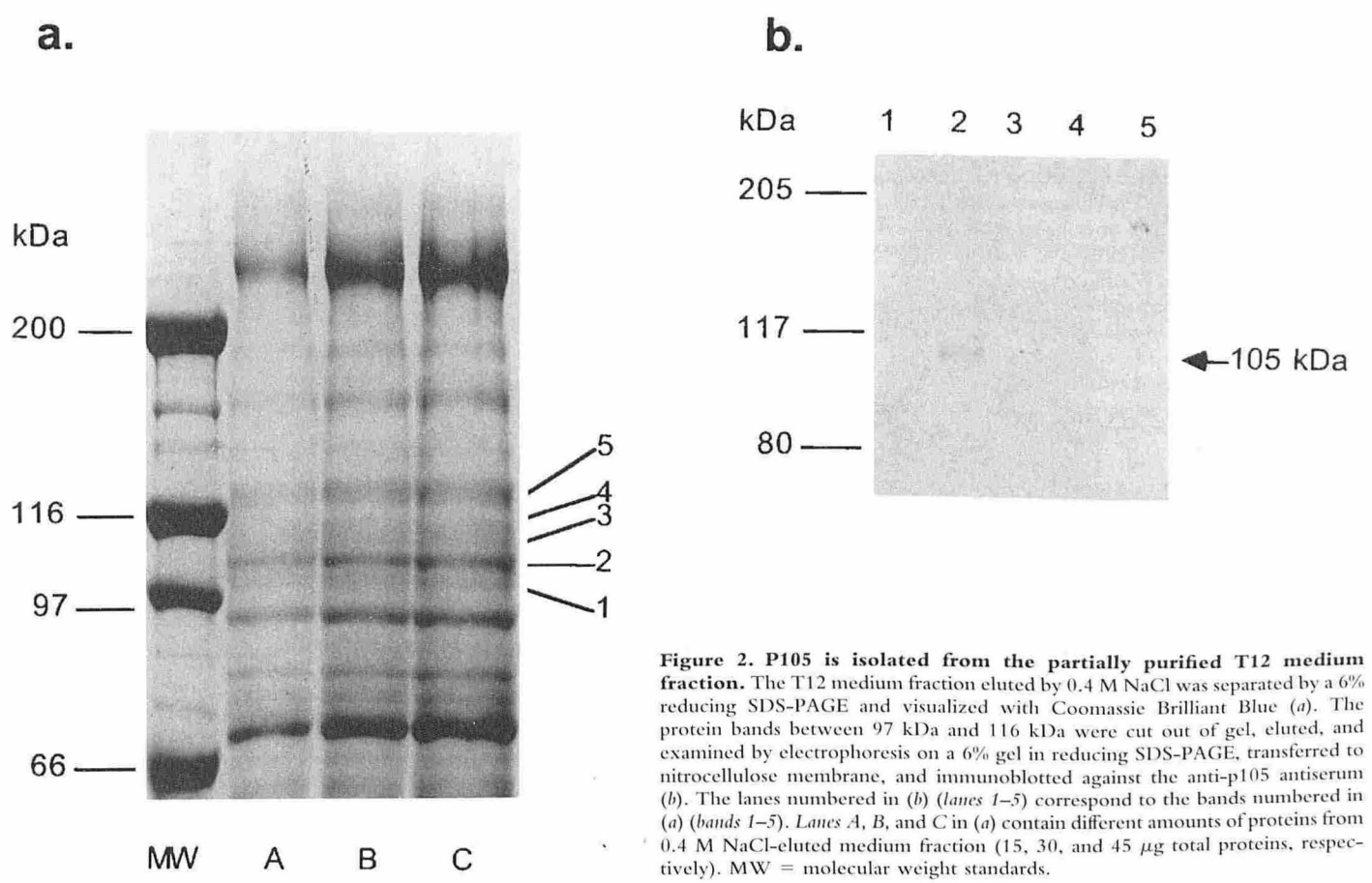

Figure 2. P105 is isolated from the partially purified T12 medium fraction. The T12 medium fraction eluted by 0.4 M NaCl was separated by a 6% reducing SDS-PAGE and visualized with Coomassie Brilliant Blue (*a*). The protein bands between 97 kDa and 116 kDa were cut out of gel, eluted, and examined by electrophoresis on a 6% gel in reducing SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted against the anti-p105 antiserum (*b*). The lanes numbered in (*b*) (*lanes 1–5*) correspond to the bands numbered in (*a*) (*bands 1–5*). *Lanes A, B,* and *C* in (*a*) contain different amounts of proteins from 0.4 M NaCl-eluted medium fraction (15, 30, and 45 μg total proteins, respectively). MW = molecular weight standards.

Table I. P105: The First 20 *N*-Terminal Amino Acid Sequence Is 70% Homologous to a 90-kDa Tumor-Associated Antigen (TAA)

	(N)[a] (Q) (E) (A)(S)(K) (P)
P105:	*N terminus*-S-N-D-G-D-M-R-M-A-D-G-E-D-T-N-Q-G-H-V-E
TAA[b]:	*N* terminus-V-N-D-G-D-M-C-L-A-D-G-G-A-T-N-Q-G-R-V-E
	(P) (L)
L3 Antigen[c]:	*N* terminus-V-N-D-G-D-M-R-L-A-D-G-G-A-T-N-Q-G-R-V-E
Mac-2 BP[d]:	*N* terminus-V-N-D-G-G-M-R-L-A-X-X-G

[a] Parentheses indicate alternative amino acids as determined by protein microsequencing. The *N*-terminal amino acid sequence for p105 has been registered with the National Biomedical Research Foundation (Washington, DC), with accession number A55899.
[b] Iacobelli *et al* (1993).
[c] Linsley *et al* (1986).
[d] Rosenberg *et al* (1991).

whereas the p105 was readily detected in the control sample (data not shown), providing evidence that p105 was synthesized by T12 fibroblasts and not a serum component. To further confirm that p105 is a fibroblast-produced protein, we electrophoresed a small quantity of culture medium that contained DMEM, 10% fetal bovine serum, and 1% penicillin/streptomycin along with T12 conditioned medium, transblotted it to nitrocellulose membrane, and immunoblotted it against the anti-p105 antiserum. The results showed that only T12 conditioned medium, and not the culture medium, contained an immunoreactive 105-kDa band, thus further confirming that p105 is not a contaminant in the culture medium (data not shown).

Verification of the *N*-Terminal Amino Acid Sequence In order to verify that the amino acid sequence obtained by microsequencing indeed encodes for p105, a multiple antigen peptide was synthesized and was used to immunize rabbits. When immunoblotting was performed with total keratinocyte and T12 fibroblast cytosol proteins, the immune serum, but not the pre-immune serum, labeled a 105-kDa protein band in both keratinocyte cellular extracts **(Fig 5)** and T12 fibroblast cellular extracts (data not shown) that co-migrated with a 105-kDa band labeled by a reference patient's anti-p105 antiserum. In addition, the immune rabbit serum labeled a BMZ component of the monkey esophagus epithelium to a titer of 1:320 **(Fig 6)**. The immune serum labeled the BMZ only with 0.3% Nonidet P-40 (and not with 0.05% Tween-20 or 0.5% Triton X100) added to the dilution buffer (1% bovine serum albumin in Tris-buffered saline). Pre-immune serum from the same rabbit did not react with the basement membrane substrate in any of the three conditions. The immune rabbit serum did not label a BMZ component of intact or salt-split normal human skin substrate. One possible explanation is that the *N* terminus of p105 is not exposed in human skin.

DISCUSSION

When we first reported the discovery of p105 (Chan *et al*, 1993), there was a concern that p105 may actually be the 105-kDa truncated laminin-5 γ2 chain (Rousselle *et al*, 1991). As further immunologic and biochemical studies on p105 progressed, it became clear that p105 is distinct from the laminin-5 γ2 chain (Cotell *et al*, 1994; Chan *et al*, 1995a, Chan *et al*, 1995b). In this study, we provide additional biochemical data regarding p105. We now know that p105 resists digestion by some common glycosidases. In addition, we have determined that p105 contains peptide and ester bonds at the carboxylic side of Glu or both Glu and Asp.

Furthermore, we have provided amino acid data to demonstrate that p105 is a unique basement membrane component. The first 20 amino acids of p105 at the *N* terminus demonstrated no homology

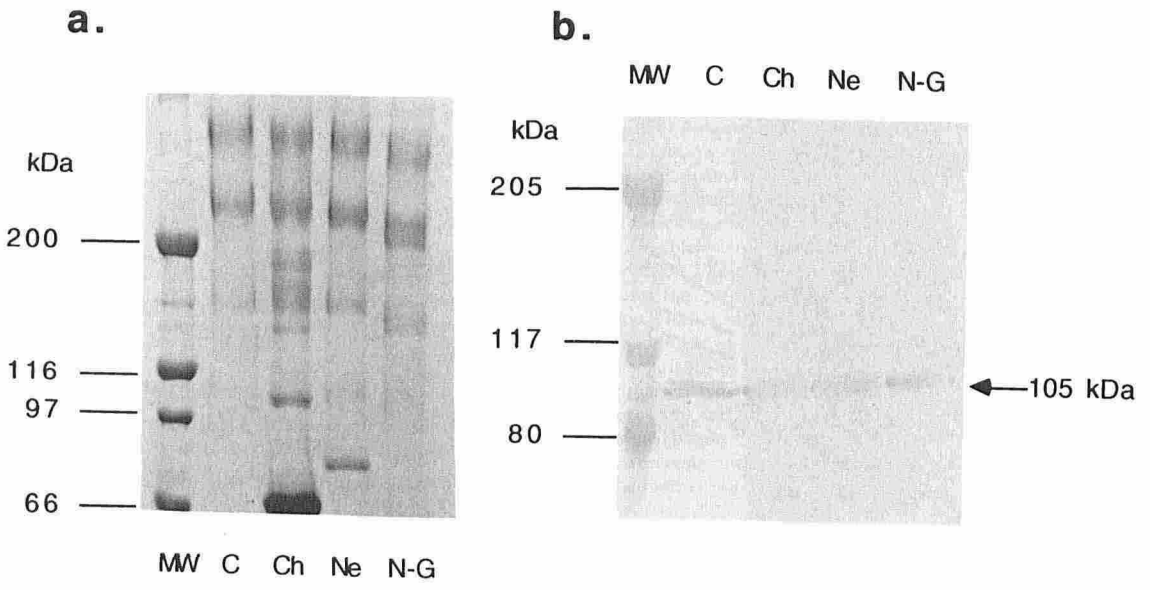

Figure 3. P105 is resistant to glycosidase digestion. The control glycoprotein laminin-1 (*a*) and the 0.4 M NaCl-eluted fraction of T12 medium (*b*) were either undigested (*lane C*) or digested with chondroitinase ABC (*lane Ch*), neuraminidase (*lane Ne*), or N-glycosidase F (*lane N-G*). The undigested samples and digested products were electrophoresed on a 6% gel in reducing SDS-PAGE and visualized with Coomassie Brilliant Blue (*a*, laminin-1) or by transferring to nitrocellulose membrane and immnoblotting against the anti-p105 antiserum (*b*, T12 medium).

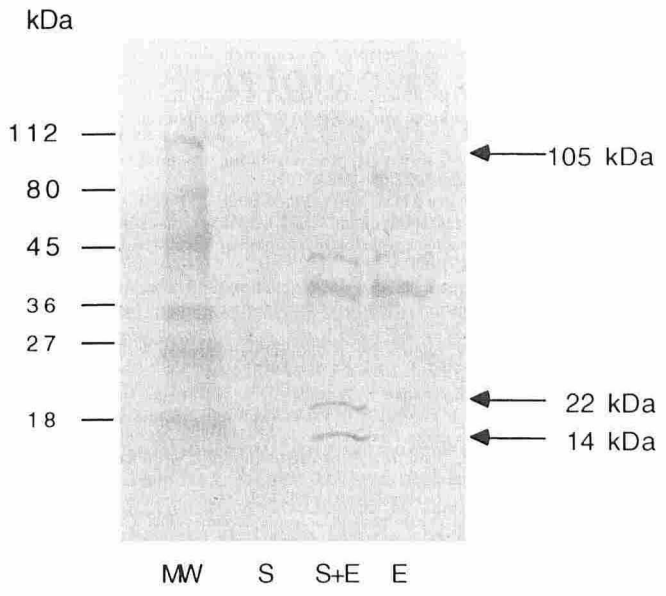

Figure 4. P105 consists of two proteolytically stable antigenic fragments. The 0.4 M NaCl-eluted T12 medium was digested with staphylcoccal protease V8, electrophoresed on a 4–20% gradient gel, transferred to a nitrocellulose membrane, and immunoblotted against the anti-p105 antiserum. *Lane S* is the control sample without enzyme digestion. *Lane S+E* is enzyme-digested sample. *Lane E* is enzyme alone. MW = molecular weight standards.

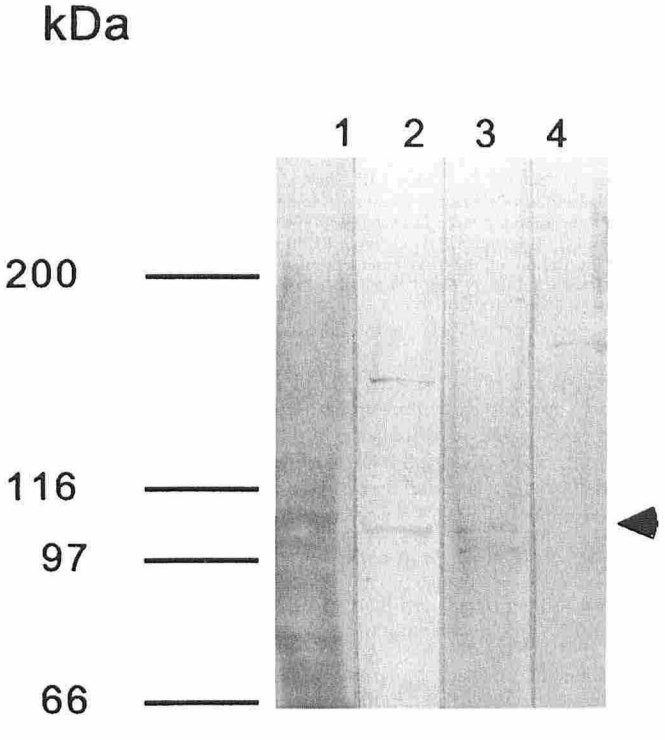

Figure 5. Polyclonal anti-peptide antibody reacted with a 105-kDa keratinocyte protein band that co-migrated with p105. Total keratinocyte cytosol proteins were electrophoresed on a 6% gel in reducing SDS-PAGE, transferred to a nitrocellulose membrane, and stained with Amido black (*lane 1*), or immunoblotted against pre-immune rabbit serum (*lane 4*), a polyclonal rabbit antibody raised against the synthetic peptide generated from the microsequenced N-terminal amino acids (*lane 3*), or a reference patient serum containing anti-p105 antiserum (*lane 2*).

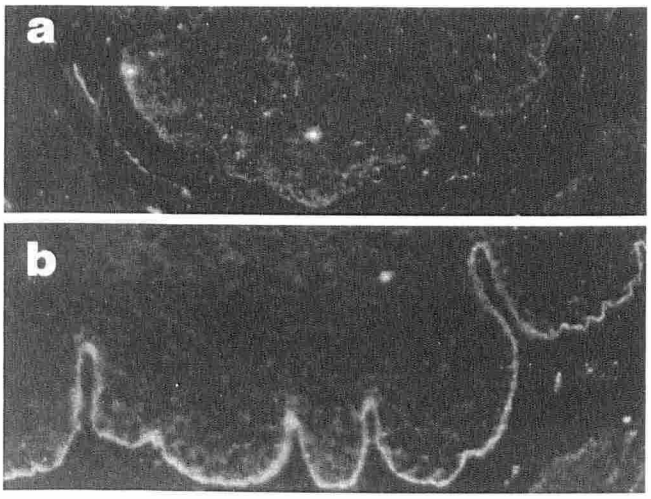

Figure 6. Polyclonal anti-peptide antibody labels an epithelial basement membrane component. Monkey esophagus was reacted with either pre-immune rabbit serum (*a*) or rabbit polyclonal antibody raised against the synthetic peptide generated from the microsequenced *N*-terminal amino acids in the presence of 0.3% Nonidet P-40 (*b*) and visualized with fluorescein-labeled goat anti-rabbit IgG.

to other known BMZ proteins when the sequence was compared in the Genbank data base. It has 70% homology to a TAA, not known to be present in skin basement membrane (Linsley *et al*, 1986; Rosenberg *et al*, 1991; Iacobelli *et al*, 1993). Unlike TAA, a glycoprotein, and laminin-1, our control glycoprotein, which are sensitive to digestion by neuraminidase and N-glycosidase F (Linsley *et al*, 1986; Rosenberg *et al*, 1991), p105 is resistant to both. Unlike TAA, which has two distinct molecular sizes for the cellular form (76-kDa) and the secreted form (94-kDa) (Linsley *et al*, 1986), p105 has the same molecular size in both cellular and secreted forms (Chan *et al*, 1993; Cotell *et al*, 1994). This indicates that there is no significant carbohydrate modification of p105 during its secretion from cells.

P105's distinction from the TAA aside, the significant homology between p105 and the TAA at the *N* terminus suggests that they may have similar biologic functions. The TAA is a binding protein for the soluble lactose/galactose-specific lectin termed Mac-2 (Ho and Springer, 1982; Koths *et al*, 1993). Mac-2, in turn, is a non-integrin laminin–1-binding protein (Woo *et al*, 1990). Thus, the TAA is an indirect laminin–1-binding protein. Being homologous to the TAA and localized proximally to laminin-1 at the skin BMZ, p105 may play an indirect role in the maintenance of BMZ stability by binding other laminin-1-binding components in the BMZ, thus contributing to the adherence of the dermal-epidermal junction.

The TAA also appears to have a role in regulating tumor progression. Some tumors that express a low level of or no TAA have been shown to overexpress markers of aggressive progression and are associated with poor prognosis (Ullrich *et al*, 1994). Being homologous to the TAA and localized at the BMZ, p105 may play an important role in keeping epithelial tumors in check.

Interestingly, the TAA has been shown to be a potent immune stimulator (Ullrich *et al*, 1994). The TAA binds Mac-2, a lectin expressed by activated macrophages, and may play a role in mediating cellular immune reaction (Ho and Springer, 1982). Co-stimulation of peripheral blood leukocytes with T-cell mitogen concanavalin A and purified TAA resulted in a significant increase in interleukin-2 secretion (Ullrich *et al*, 1994). In addition, abnormal serum levels of TAA have been found in patients with two autoimmune diseases, rheumatoid arthritis and systemic lupus erythematosus (Ullrich *et al*, 1994). Being homologous to TAA, p105 can also become a potent immunogen when it is exposed to

the immune system. The fact that p105 is targeted by an autoimmune reaction supports this possibility.

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REFERENCES

Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinman H, Martin GR, Meneguzzi G, Paulsson M, Sanes J, Timpl R, Tryggvason K, Yamada Y, Yurchenco PD: A new nomenclature for the laminins. *Matrix Biol* 14:209–211, 1994

Carter WG, Ryan MC, Gahr PJ: Epiligrin, a new cell adhesion ligand for integrin $\alpha 3\beta 1$ in epithelial basement membranes. *Cell* 65:599–610, 1991

Chan LS, Cooper KD: A novel immune-mediated subepidermal bullous dermatosis characterized by IgG autoantibodies to a lower lamina lucida component. *Arch Dermatol* 130:343–347, 1994

Chan LS, Fine JD, Briggaman RA, Woodley DT, Hammerberg C, Drugge RJ, Cooper KD: Identification and partial characterization of a novel 105-kDalton lower lamina lucida autoantigen associated with a novel immune-mediated subepidermal blistering disease. *J Invest Dermatol* 101:262–267, 1993

Chan LS, Fine JD, Hammerberg C, Bauer EA, Cooper KD: Defective *in vivo* expression and apparently normal *in vitro* expression of a newly identified 105-kDa lower lamina lucida protein in dystrophic epidermolysis bullosa. *Br J Dermatol* 132:725–729, 1995a

Chan LS, Wang XS, Lapiere JC, Marinkovich MP, Jones JCR, Woodley DT: A newly identified 105-kD lower lamina lucida autoantigen is an acidic protein distinct from the 105-kD $\gamma 2$ chain of laminin-5. *J Invest Dermatol* 105:75–79, 1995b

Cotell SL, Lapiere JC, Chen JD, Iwasaki T, Krusinski PA, Chan LS, Woodley DT: A novel 105-kDa lamina lucida autoantigen: association with bullous pemphigoid. *J Invest Dermatol* 103:78–83, 1994

Diaz LA, Ratrie H, Saunders WS, Futamura S, Squiquera HL, Anhalt GJ, Giudice GJ: Isolation of a human epidermal cDNA corresponding to the 180-kD autoantigen recognized by bullous pemphigoid and herpes gestationis sera. Immunolocalization of this protein to the hemidesmosome. *J Clin Invest* 87:1088–1094, 1990

Domloge-Hultsch N, Gammon WR, Briggaman RA, Gil SG, Carter WG, Yancey KB: Epiligrin, the major human keratinocyte integrin ligand, is a target in both an acquired autoimmune and an inherited subepidermal blistering skin disease. *J Clin Invest* 90:1628–1633, 1992

Goolsby CL, Wiley JE, Steiner M, Bartholdi MF, Cram LS, Kraemer PM: Karyotype evolution in a simian virus 40-transformed tumorigenic human cell line. *Cancer Genet Cytogenet* 49:231–248, 1991

Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill JS, Vorhees JJ, Cooper KD: Interleukin-1 receptor antagonist in normal and psoriatic epidermis. *J Clin Invest* 90:571–583, 1992

Ho MK, Springer TA: Mac-2, a novel 32,000 Mr mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. *J Immunol* 128:1221–1228, 1982

Iacobelli S, Bucci I, D'Egidio M, Giuliani C, Natoli C, Tinari N, Rubistein M, Schlessinger J: Purification and characterization of a 90-kDa protein released from human tumor and tumor cell lines. *FEBS Lett* 319:59–65, 1993

Koths K, Taylor E, Halenbeck R, Casipit C, Wang A: Cloning and characterization of a human Mac-2-binding protein, a new member of the superfamily defined by the macrophage scavenger receptor cysteine-rich domain. *J Biol Chem* 268: 14245–14249, 1993

Labib RS, Anhalt GJ, Patel HP, Mutasim DF, Diaz LA: Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 136: 1231–1235, 1986

Laemmli UK: Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970

Leigh IM, Eady RAJ, Heagerty AHM, Purkis PE, Whitehead PA, Burgeson RE: Type VII collagen is a normal component of epidermal basement membrane which shows altered expression in recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 90:639–642, 1988

Linsley PS, Horn D, Marquardt H, Brown JP, Hellstrom I,Hellstrom K-E, Ochs V, Tolentino E: Identification of a novel serum protein secreted by lung carcinoma cells. *Biochemistry* 25:2978–2986, 1986

Mueller S, Klaus-Kovtun V, Stanley JR: A 230-kD basic protein is the major bullous pemphigoid antigen. *J Invest Dermatol* 92:33–38, 1989

Parente MG, Chung LC, Ryynanen J, Woodley DT, Wynn KC, Bauer EA, Mattei MG, Chu ML, Uitto J: Human type VII collagen: cDNA cloning and chromosomal mapping of the gene. *Proc Natl Acad Sci USA* 88:6931–6935, 1991

Posnett DN, McGrath H, Tam JP: A novel method for producing anti-peptide antibodies. *J Biol Chem* 263:1719–1725, 1988

Rosenberg I, Cherayil BJ, Isselbacher KJ, Pillai S: Mac-2-binding glycoproteins: putative ligands for a cytosolic β-galactoside lectin. *J Biol Chem* 266:18731–18736, 1991

Rousselle P, Lunstrum GP, Keene DR, Burgeson RE: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filament. *J Cell Biol* 114:567–576, 1991

Ryynanen M, Ryynanen J, Sollberg S, Iozzo RV, Knowlton RG, Uitto J: Genetic linkage of type VII collagen (COL7A1) to dominant dystrophic epidermolysis bullosa in families with abnormal anchoring fibrils. *J Clin Invest* 89:974–980, 1992

Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI: Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. *Cell* 24:897–903, 1981

Stanley JR, Tanaka T, Mueller S, Klaus-Kovtun V, Roop D: Isolation of complementary DNA for bullous pemphigoid antigen by use of patient's autoantibodies. *J Clin Invest* 82:1864–1870, 1988

Tam JP: Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc Natl Acad Sci USA* 85:5409–5413, 1988

Uitto J, Christiano AM: Molecular genetics of the cutaneous basement membrane zone. perspectives on epidermolysis bullosa and other blistering skin disease. *J Clin Invest* 90:687–692, 1992

Ullrich A, Sures I, D'Egidio M, Jallal B, Powell TJ, Herbst R, Dreps A, Azam M, Rubinstein M, Natoli C, Shawver LK, Schlessinger J, Iacobelli S: The secreted tumor-associated antigen 90K is a potent immune stimulator. *J Biol Chem* 269:18401–18407, 1994

Woo HJ, Shaw LM, Messier JM, Mercurio AM: The major non-integrin laminin-binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). *J Biol Chem* 265:7097–7099, 1990

Woodley D, Sauder D, Talley MJ, Silver M, Grotendorst G, Qwarnstrom E: Localization of basement membrane components after dermal-epidermal junction separation. *J Invest Dermatol* 81:149–153, 1983

Woodley DT, Briggaman RA, O'Keefe EJ, Inman AO, Queen LL, Gammon WR: Identification of the skin basement membrane autoantigen in epidermolysis bullosa acquisita. *N Engl J Med* 310:107–113 1984