Characterization of a 50-kDa Component of Epithelial Basement Membranes Using GDA-J/F3 Monoclonal Antibody*

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Using the monoclonal antibody GDA-J/F3, a 50-kDa noncollagenous component of human skin basement membrane zone was identified. Immunofluorescence stainings of normal human skin with the GDA-J/F3 antibody showed a linear fluorescence decorating the basement membrane zone. With immunoelectron microscopy, the epitope was localized to the insertion points of the anchoring fibrils into the lamina densa. The antigen is distinct from collagen VII, from the main structural protein of the anchoring fibrils, and from several other structural molecules of the basement membrane zone, because the GDA-J/F3 antibody did not react with purified basement membrane components in vitro. In serum-free cultures, the antigen was synthesized and secreted by normal and transformed human keratinocytes and fibroblasts. Immunoprecipitation of radiolabeled epithelial cell-conditioned medium with the GDA-J/F3 antibody yielded two polypeptides that migrated on SDS-polyacrylamide gel electrophoresis with apparent molecular masses of 46 and 50 kDa under nonreducing conditions. Using reducing gels, only the 50-kDa polypeptide was observed. The antigen was resistant to digestion with bacterial collagenase but sensitive to trypsin and pepsin. It also bound to heparin and DEAE cellulose at low ionic strength and alkaline pH. These findings indicate that the GDA-J/F3 antigen is a small globular disulphide-bonded protein with a potential to interact with basement membrane proteoglycans. Integration of the GDA-J/F3 antigen into the histarchitecture of the dermo-epidermal junction is dependent on the presence of collagen VII, because the GDA-J/F3 epitope was missing in several patients with a genetic blistering disorder of the skin, epidermolysis bullosa dystrophica, who lacked collagen VII and anchoring fibrils.

The basement membrane zone of human skin represents a highly specialized epithelial-mesenchymal interface. Its major function is to attach the skin layers, the epidermis and the dermis, to each other and to provide resistance against external shearing forces. The zone consists of three supramolecular networks, the hemidesmosome-anchoring filament complex, the basement membrane itself, and the anchoring fibrils. The first network binds the epidermal keratinocytes to the basement membrane, and the anchoring fibrils connect the basement membrane with the dermis (1). The morphological structure of the dermo-epidermal junction zone is well known from electron microscopic studies (2), and many of the most abundant molecular components have been characterized at protein and cDNA levels (for reviews see Refs. 3–6). Important adhesive molecules are the bullous pemphigoid antigens BP230 and BP180, the α6β4 integrin, and laminin 5 and 6 in the hemidesmosome-anchoring filament complex, collagen IV, nidogen and perlecan in the basement membrane itself, and collagen VII in the anchoring fibrils. All these molecules have a modular structure with typical domains of adhesive matrix components, and they are strongly interactive with multiple binding sites through which they contribute to the dermo-epidermal cohesion by chemical and physical interactions (5).

Many basement membrane macromolecules are target proteins in both hereditary and acquired blistering diseases of the skin (2, 7). Their impaired structure and function lead to diminished cohesion of the skin layers in these disorders. In hereditary epidermolysis bullosa (EB)1 mechanical trauma induces detachment of the epidermis from the dermis. Mutations in the genes encoding BP230 (8), BP180 (9, 10), α6β4 integrin (11), laminin 5 (12, 13), and collagen VII (14, 15) have been defined in different EB subgroups. Interestingly, the same structural macromolecules are targets in many acquired autoimmune disorders. Patients with different autoimmune blistering disorders can have tissue bound and circulating autoantibodies to BP230, BP180, laminin 5, or collagen VII (16–18).

In addition, hitherto unknown molecular components of the skin basement membrane zone are likely to exist. The fact that many patients with genetic diseases affecting the dermal-epidermal junction do not have mutations in the known candidate genes supports this notion. Furthermore, several monoclonal antibodies raised to extracts of the basement membrane zone have disclosed novel distinct epitopes that have not yet been biochemically characterized, such as AF1, AF2 (19), or DEJ-19–1 (20).

The monoclonal antibody GDA-J/F3 was raised against ejaculated human spermatozoa and their precursor cells (21). Unexpectedly, this antibody not only recognized the sperm tails but also strongly reacted with the epithelial-mesenchymal basement membrane of the skin, trachea, ureter, and amnion.

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1 The abbreviations used are: EB, epidermolysis bullosa; IF, immunofluorescence; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis.
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Indirect Immunofluorescence Staining—IF staining was carried out as described previously (27). The primary antibodies were used at 1:200 dilution, and the secondary antibodies were used at 1:200 dilution. The slides were examined with a Zeiss epifluorescence microscope.

Inmunoprecipitation—Subconfluent cells were grown for 24 h in Met/Cys-free Dulbecco’s modified Eagle’s medium supplemented with 40 μCi/ml of [35S]Cys/Met mixture (ICN Biomedicals, Orsay, France) and 50 μg/ml ascorbic acid. All subsequent steps were performed at 4 °C. The medium was collected, supplemented with proteinase inhibitors, and centrifuged at 1200 rpm for 5 min. The cell layer was washed twice with cold PBS, and the cells were scraped with a rubber policeman into PBS containing the above proteinase inhibitors. 1 ml of PBS was used per 75 cm² flask. The cells were homogenized with a Potter homogenizer, stirred for 1 h, and centrifuged at 13,000 rpm for 30 min. The cell supernatant was used for immunoprecipitation (30). 50 μl of protein A-Sepharose (Pharmacia Biotech Inc., Freiburg, Germany) was preincubated with 10 μg of rabbit-anti-mouse IgG (Dako) overnight, washed with PBS three times, and incubated for 3 h with 50 μl of GDA-J/F3 antibody supernatant or 1 μl of antibody ascites or with 60 μl of BM 165 supernatant. In negative controls, the GDA-J/F3 or BM 165 antibody was omitted. The protein A-antibody complexes were washed three times with PBS and incubated with 500 μl of [35S]labeled medium or 500 μl of cell lysate for 1 h. The protein A-Sepharose immune complexes were washed four times with 0.05% Tween 20 in PBS and twice with PBS-2 min centrifugations at 3000 rpm. The immune complexes were dissociated by boiling for 5 min in SDS-PAGE sample buffer (31).

For pulse-chase experiments, subconfluent cells were metabolically labeled with 40 μCi/ml of [35S]Cys/Met mixture (ICN Biomedicals, Orsay, France) for 30 min and chased with nonradioactive medium for 0 min, 30 min, 90 min, 3 h, 6 h, and 24 h. Thereafter the medium and the cell layer were processed for immunoprecipitation as described above.

In some experiments, the immunoprecipitation products obtained with the GDA-J/F3 antibody from medium of SCC25 cells were separated by SDS-PAGE with a 4.5–15% polyacrylamide gradient. The bands of 46 and 50 kDa were excised from the gel and reduced with 5% β-mercaptoethanol in Laemmli sample buffer for 5 min at 95 °C. The samples were then separately reloaded onto SDS-PAGE with a 4.5–15% polyacrylamide gradient gel.

Proteinase Digestions—500 μl of [35S]-labeled medium was treated with 10 μg/ml trypsin (Seronema/Biochrom, Strasbourg, France) for 4 h at 37 °C or with 100 μg/ml papain (Serva, Saint-Germain-en-Laye, France) in 0.1 M acetic acid overnight at 4 °C. Collagenase digestion was carried out with 20 μg/ml of purified bacterial collagenase from Clostridium histolyticum (Warthington CLSFA, Pharmacaure, Brugg, Switzerland) in the presence of CaCl₂ and 10 mM N-ethylmaleimide for 4 h at 39 °C. The enzyme activities were terminated with 10 μg/ml soya bean trypsin inhibitor, by neutralizing the pH, or with 10 mM EDTA, respectively. In negative controls, the enzyme was omitted from the incubation mixture. As a positive control, 10 μg of fetal bovine collagen I (kindly provided by Dr. D. Herbager, Institute de Biologie et Chimie des Protéines, Lyon, France) were subjected to collagenase digestion.

Detection of the GDA-J/F3 Antigen by Dot Blotting Assays—Aliquots of serum-free cell culture medium, column chromatography fractions, or 0.1–5 μg of purified extracellular matrix or basement membrane components were dotted onto nitrocellulose filters using a vacuum manifold (32). After blocking with 2%–4% milk protein in Tris-buffered saline, the filters were incubated with the GDA-J/F3 antibody followed by peroxidase-conjugated secondary antibodies (Boehringer Mannheim, Meylan, France). Bound antibodies were revealed using the BM Chemiluminescence kit (Boehringer Mannheim) or a semiquantitative immunoblot assay as described previously (27).

The matrix proteins tested for reactivity with the GDA-J/F3 antibody in these assays were the following: NC-1 domain of human collagen VII, isolated with collagenase digestion from amnion (Ref. 33; kind gift of Dr. Patricia Rousselle, Institut de Biologie et Chimie des Protéines, Lyon, France); triple helical domain of human collagen VII, isolated with papain digestion from amnion (Ref. 25); procarboxylic (15) and eucarboxy recombinant NC-2 domain of human collagen VII (kindly provided by Dr. E. Kohfeldt, Max-Planck-Institute, Martinsried, Germany); triple helical domain of human collagen V, isolated with papain digestion from amnion (28); bovine skin collagen I (kindly provided by Dr. D. Herbager, Institute de Biologie et Chimie des Protéines, Lyon, France); human fibronectin (Sigma); human vitronectin (Life Technologies, Inc.); recombinant human perlecan (kind gift of Dr. Rupert Timpl, Max-Planck-Institute, Martinsried, Germany); PRELP (Ref. 35, kind gift of Dr. Patricia Rousselle, Institut de Biologie et Chimie des Protéines, Lyon, France); human fibronectin (Sigma).
RESULTS

Identification of the GDA-J/F3 Antigen—The GDA-J/F3 antibody did not recognize antigens in immunoblots (22), presumably due to denaturation of the epitopes during SDS-PAGE and electrotransfer. Application of “denaturing and partially renaturing” steps with decreasing concentrations of urea or guanidine HCl (36, 37) after SDS-PAGE and electrotransfer to nitrocellulose did not restore reactivity of skin or cell extracts with GDA-J/F3 (data not shown). However, dot binding assays showed the presence of the GDA-J/F3 antigen in culture medium of primary human keratinocytes, HaCaT and SSC25 cells (Fig. 1), and several other epithelial cell lines as well as human skin fibroblasts (not shown), the best producers being the epithelial cell lines. Exposing SCC25 cell culture medium to low concentrations of denaturing or non-denaturing detergents like 0.1% SDS or 0.1% Triton X-100 resulted in a loss of antigen-antibody interactions (Fig. 1), a property that precludes investigations based on SDS-PAGE and immunoblotting of tissue or cell culture extracts. To overcome this problem, we adopted immunoprecipitation techniques that proved useful in recovering the antigen from the culture medium of metabolically labeled cells or from cell lysates. The GDA-J/F3 antibody immunoprecipitated from keratinocyte medium a protein with an apparent molecular mass of 50 kDa on reducing SDS-PAGE (Fig. 2). However, the quantity of the protein in the culture medium was significantly lower than that of laminin 5, another secreted keratinocyte product. In nonreducing SDS gels, two bands with apparent molecular masses of 46 and 50 kDa were observed (Fig. 3A, lane 3). The intensity of the two bands precipitated from cell culture medium was approximately equal, whereas in cell lysates the 50-kDa band appeared more dominant (Fig. 3A). Upon reduction, only the 50-kDa band was seen (Fig. 3A, lane 4). To determine the relationship of the bands, immunoprecipitates were separated using nonreducing SDS-PAGE, and the 50- and 46-kDa bands were excised from the gel. After reduction with 5% β-mercaptoethanol, the bands were separately reloaded on SDS-PAGE. As shown in Fig. 3B (lane 3), the 46-kDa band now migrated with an apparent molecular mass of 50 kDa; this shift suggested the presence of intramolecular disulfide bonds. This was in contrast to the other 50-kDa polypeptide whose mobility was not affected by the reducing agent.

In some experiments, immunoprecipitated large molecular weight material was observed on the top of the gel. To investigate the possibility that this material represented a precursor...
form of the 50-kDa protein, pulse-chase experiments were carried out. The cells were pulsed with medium containing \(^{35}\text{S}\)Cys/Met for a short period of time (30 min), then chased with nonradioactive medium for 30 min, 90 min, 3 h, 6 h, and 24 h, and subsequently immunoprecipitated (Fig. 3C). Both the 50-kDa and the 46-kDa bands, but no large molecular mass material, were detected in the cell lysates, indicating absence of a precursor form. Also the fact that the 50- and 46-kDa bands were detected in both the cell extracts and the medium suggested that the protein does not undergo major post-translational processing. Therefore, it is likely that the material observed at the top of gels of some immunoprecipitated medium samples represented specific or nonspecific aggregates of the GDA-J/F3 antigen with itself or with other medium molecules.

Digestion of metabolically labeled conditioned medium with purified \(C.\ histolyticum\) collagenase for 4 h at 39 °C did not result in degradation of the GDA-J/F3 antigen, indicating lack of collagen-like structural domains (Fig. 4A). Under the same conditions, collagen I was completely digested by collagenase. In contrast, the GDA-J/F3 antigen was sensitive to treatment with trypsin in neutral or pepsin in an acid pH, respectively, suggesting a globular structure of the protein (Fig. 4A). The 50-kDa and the 46-kDa band behaved similarly during digestion, both being sensitive to trypsin and pepsin and resistant to collagenase.

The GDA-J/F3 antigen was endowed with the property to bind DEAE-cellulose at low ionic strength at pH 8.5, and it could be eluted from the column with 0.13–0.20 M NaCl as shown by screening the eluted fractions with a dot binding immunooassay (Fig. 5). However, as indicated by the absorbance at 280 nm of the chromatogram, it represented a very small fraction of the bound material from the culture medium. Correspondingly, SDS-PAGE analysis of the GDA-J/F3 antigen containing fractions revealed the presence of several other polypeptides in Coomassie Blue staining (not shown).

Another biochemical feature of the GDA-J/F3 antigen was its affinity to heparin. It was possible to remove the antigen from HaCaT cell culture medium with chromatography on heparin-Sepharose under physiological salt concentration and neutral pH. Some antigen leaked from the column during washing with PBS, but the majority eluted with 0.5 M NaCl at pH 8.5 (Fig. 6). This result implied binding of the GDA-J/F3 antigen to heparin on the basis of ionic interactions. Analysis on SDS-PAGE of the proteins eluted from the heparin column revealed after Coomassie Blue staining a number of bands, indicating that the GDA-J/F3 antigen was one of many heparin-binding molecules in the medium of epithelial cells.

The antigenic relationship of the GDA-J/F3 epitope with other components of the basement membrane region that might give rise to a 50-kDa noncollagenous fragment was addressed with immunoprecipitation reactions and with native dot binding assays. In immunoprecipitates of cell extracts and culture media with the GDA-J/F3 antibody, only the 46- and 50-kDa bands were excised from the gel, incubated with 5% \(-\text{mercaptoethanol}\) containing sample buffer, and rerun on an SDS-PAGE with a 4.5–15% polyacrylamide gradient. Lane 2, the initial 50-kDa band. Lane 3, the initial 46-kDa band. After reduction, the 46-kDa band migrated with an apparent molecular mass of 50-kDa, whereas the mobility of the initial 50-kDa band was not affected. C, pulse-chase experiment with the cell layer to investigate presence of precursor forms of the 46- and 50-kDa bands. After the chase the cells were extracted and immunoprecipitated. The products were separated on SDS-PAGE with a 4.5–15% polyacrylamide gradient under nonreducing conditions. Lane 7 shows a control with cells labeled for 24 h, similar to lane 7 in A. No evidence for larger precursor forms in the cells was found. \(-\text{ME}, \text{\text{-mercaptoethanol}}\).
50-kDa bands were observed, and no other bands with masses corresponding to known components of the basement membrane or the skin extracellular matrix (see Figs. 2 and 3, A and C). Moreover, 0.1–5 μg of collagens I, IV, and V, different placental laminins, fibronectin, vitronectin, the 58-kDa proteoglycan PRELP, and recombinant perlecain were all reacted with GDA-J/F3 antibody. However, none of them was recognized by it (not shown).

Because the GDA-J/F3 antigen was localized in close vicinity of collagen VII in situ, the reactivity of the antibody with purified human collagen VII and its fragments was carefully investigated. Purified, native human procollagen VII fragments were used as antigens in dot blot assays. For this purpose, the NC-1 domain was isolated with collagenase digestion (33), and the triple helical domain with pepsin digestion (25) from human amnion. Recombinant NC-2 domain was produced using both prokaryotic and eukaryotic expression systems (15). None of these collagen VII fragments reacted with the GDA-J/F3 antibody. Furthermore, procollagen VII could not be immunoprecipitated from keratinocyte cultures with the GDA-J/F3 antibody. However, none of them was recognized by it (not shown).

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None of these collagen VII fragments reacted with the GDA-J/F3 antibody. Furthermore, procollagen VII could not be immunoprecipitated from keratinocyte cultures with the GDA-J/F3 antibody (see Figs. 2 and 3, A and C). All these findings are consistent with the assumption that the GDA-J/F3 antigen is distinct from collagen VII.

Expression of the GDA-J/F3 Antigen in Cultured Cutaneous Cells—Studies on in vitro expression of GDA-J/F3 antigen were carried out using indirect IF staining of cultured cutaneous cells. Normal epidermal keratinocytes (Fig. 7), the spontaneously transformed epidermal HaCaT cells, the skin carcinomas derived cell line SCC25, the basement membrane producing cell lines HT 1080 and HBL 100, and epithelial cells derived from a benign basement membrane-rich skin tumor, cylindroma, exhibited a strong staining with the GDA-J/F3 monoclonal antibody, whereas human skin fibroblasts showed lower expression of the antigen. The expression of the GDA-J/F3 antigen seemed dependent on cell density, the proportion of cells stained increased with higher cell number. In subconfluent keratinocytes, the staining pattern consisted mainly of an intracellular granular reaction (Fig. 7, a and c), but in confluent cultures also a dotty extracellular staining was observed (Fig. 7, b and d). In comparison, antibodies to other keratinocyte products, such as laminin 1 and 5 or collagen IV and VII produced a different IF pattern. Both laminins were deposited on the surface of the culture flask and could be observed as a pericellular staining (Fig. 7, e and f). Collagen IV showed a granular intracellular and a weak extracellular staining, and collagen VII showed a granular intracellular staining (Fig. 7, g and h).

Localization of the GDA-J/F3 Antigen in the Skin—It has been shown previously that in IF staining the GDA-J/F3 monoclonal antibody reacted with the basement membrane zone of
lar space. Staining with antibodies to collagen IV (g) produced an intracellular granular staining. (h) Sarcoma laminin (e) stained the cells with antibodies to laminin 5 (a–d). In subconfluent cultures, the intracellular staining of normal keratinocytes (e–h) revealed gold particles consistently localized at the lower border of the lamina densa (Fig. 9, a–c). No staining was observed within the lamina lucida, in the hemidesmosomes, along the cross-striated section of the anchoring fibrils, or in the papillary connective tissue. These results establish GDA-J/F3 antigen as a component of the dermo-epidermal junction zone that is localized at the lamina densa region, in close vicinity of the fanning ends of the anchoring fibrils.

DISCUSSION

In the present study we characterized the GDA-J/F3 antigen, a noncollagenous protein with an apparent molecular mass of 50 kDa at the basement membrane zone of human skin. The migration of the molecule on SDS-PAGE varied upon reduction and upon solubilization with 1 M NaCl along the lamina lucida (29), the GDA-J/F3 antibody stained the blister base (Fig. 8a), indicating that the epitope was localized at or below the lamina densa of the basement membrane. In the skin of patients with localized dystrophic EB, genetic disorder with sub-lamina densa blistering, production of the GDA-J/F3 antigen was not affected, and the positive immunoreactivity remained in the blister roof (Fig. 8b) similar to staining of collagen VII (Fig. 8c), further demonstrating that the epitope was present in the lamina densa or in association with the anchoring fibrils. Yet another genetic disease could be used as a model in this context: a certain subset of severe EB, the EB dystrophica mutilans, that is characterized by lack of collagen VII and anchoring fibrils from the skin. In the skin of 11 such patients studied here, GDA-J/F3 antigen was absent as determined by IF labeling (Fig. 8e). This observation was consistent with the association of the GDA-J/F3 antigen with the anchoring fibrils.

Results of immunogold labeling of normal human skin were in concert with the above findings. Immunoelectron microscopy revealed gold particles consistently localized at the lower border of the lamina densa (Fig. 9a–c), predominantly at the junctions of the anchoring fibrils with the lamina densa (Fig. 9, b and c). No staining was observed within the lamina lucida, in the hemidesmosomes, along the cross-striated section of the anchoring fibrils, or in the papillary connective tissue. These results establish GDA-J/F3 antigen as a component of the dermo-epidermal junction zone that is localized at the lamina densa region, in close vicinity of the fanning ends of the anchoring fibrils.
FIG. 9. Ultrastructural immunolocalization of the GDA-J/F3 antigen in normal human skin. The antigen was localized with immuno electron microscopy using a silver enhanced 1-nm gold-conjugated secondary antibody. A, the gold particles appeared along the lower border of the lamina densa (LD). B and C, higher magnifications showed the GDA-J/F3 antigen localized at the insertion points of the anchoring fibrils (AF) to the lamina densa. No labeling was found in the lamina lucida (LL), hemidesmosomes (HD), or anchoring filaments (AF). Bars: in A, 500 nm; in B, 150 nm; C, 100 nm. Magnification: in A, \( \times37,500 \); B, \( \times96,000 \); C, \( \times138,000 \).

The antigen was localized with immunoblotting of skin or cell extracts for determining the presence of larger aggregates with interchain or intermolecular disulphide bonds.

Salt splitting of normal skin and natural blistering in genetically altered skin demonstrated that the GDA-J/F3 antigen was localized to the lower lamina densa of the basement membrane, in close conjunction with the anchoring fibrils. With immunoelectron microscopy, we localized the epitopes almost exclusively to the insertion points of the anchoring fibrils and the lamina densa. In previous ultrastructural studies, normal human skin was not examined, but the GDA-J/F3 antibody was used to stain oral mucosa or blistered skin of EB patients, and a more general association of the antigen with the lamina densa and the anchoring fibrils was observed (22, 38). The previous and the present findings could imply reactivity of the antibody with an already known lamina densa component or with collagen VII, the main structural protein of the anchoring fibrils. This appears very unlikely for two reasons. First, the GDA-J/F3 antibody did not react in immunoprecipitation and in native dot blot assays with a number of known components of basement membranes or extracellular matrices. Second, particular care was taken to examine and exclude cross-reactivity with collagen VII. All three structural domains of procollagen VII, the 145-kDa amino-terminal NC-1, the 20–30-kDa carboxyl-terminal NC-2, and the 170-kDa central triple helical domain were isolated and tested as antigens in native dot blot assays, but none showed reactivity with the GDA-J/F3 antibody. Further, immunoprecipitations of extracts or culture media of different epithelial cells with the GDA-J/F3 antibody never yielded procollagen VII or any of its specific fragments. These observations are consistent with the fact that the physiological processing of procollagen VII to collagen VII does not produce a 50-kDa fragment but a \( \sim 20–30 \)-kDa cleavage product from the NC-2 domain (1, 15). Moreover, the primary structure of collagen VII does not contain regions that are likely to be subjected to unspecific proteolysis and yield a 50-kDa degradation product (39).

Other small globular molecules of the basement membranes, such as the 40-kDa BM-40 or SPARC (40), the 58-kDa PRELP (35), or the 58-kDa TIN antigen (41, 42), exhibit a distinct tissue distribution and different biochemical characteristics from the GDA-J/F3 antigen. As has been demonstrated by Jassim et al. (22), and in the present investigation, the GDA-J/F3 antigen is expressed only in the sperm and under stratified epithelia in the skin, esophagus, trachea, ureter, and the placental membranes. In contrast, BM-40 is found also in vascular and kidney basement membranes, PRELP is in a number of tissues including bone and cartilage, and the TIN antigen is in the kidney tubular basement membrane as well as in the Bowman’s capsule (43). Using a monoclonal antibody to TIN, we showed that in NaCl-split normal human skin TIN was detected in the blister roof, indicating that it is localized at or above the lamina lucida.4 In contrast, the GDA-J/F3 antigen was separated into the blister base (Fig. 8a). All these data suggest that the GDA-J/F3 antigen is distinct from known basement membrane components of a similar molecular weight. The fact that the GDA-J/F3 antigen binds to heparin-Sepharose suggests that it can interact with heparin or heparan sulfate proteoglycans in vitro, such as perlecan in the lamina densa of the basement membrane or syndecan on keratinocyte cell surface, thereby contributing to cell-matrix interactions and dermal-epidermal cohesion in the skin.

A current opinion prevails that in addition to collagen VII, the anchoring fibrils contain other structural components (1). Despite the fact that the novelty of the GDA-J/F3 antigen has not been definitively established, it seems likely that the GDA-J/F3 antigen represents a distinct entity that is associated with the anchoring fibrils. IF staining of the skin of patients suffering from severe mutilating dystrophic epidermolysis bullosa supports this notion. These individuals represent nullizygotes, they lack collagen VII from the skin due to mutations leading to premature termination codons in the COL7A1 gene and therefore cannot form anchoring fibrils (14, 44). In our hands, staining with the GDA-J/F3 antibody was negative or greatly reduced in the skin of 11 such individuals, suggesting that when anchoring fibrils are absent, the GDA-J/F3 antigen cannot assemble correctly and remain stable.

In contrast to previous findings (22), we now demonstrated that normal human keratinocytes and to a significantly lesser extent human fibroblasts synthesize the GDA-J/F3 antigen in vitro. Many, but not all, structural components of the skin basement membrane zone are synthesized predominantly by epidermal cells. For example, the laminins 5 and 6 in the lamina lucida are epithelial products (45). Also collagen VII is synthesized by epidermal keratinocytes in vitro and in vivo, even though the anchoring fibrils are localized at the dermal

4 L. Bruckner-Tuderman and R. Butkowski, unpublished observation.
side of the basement membrane zone (27, 46). In contrast, nidogen is solely a product of dermal fibroblasts (47), indicating complex synthetic pathways and intermolecular interactions for the formation of a functional basement membrane zone in the skin. Quantitatively, the GDA-J/F3 antigen is a very minor biosynthetic product of cultured epithelial cells. Comparison of GDA-J/F3 antigen expression to that of other keratinocyte products, collagen VII and laminin 5 in IF or immunoprecipitation experiments (Figs. 2 and 7) demonstrated that GDA-J/F3 antigen is expressed at a remarkably lower level than the two other proteins. In this context it may be of interest to stress that previous studies have estimated that collagen VII represents less than 0.001% of total extractable skin collagen (25). The combination of minimal quantities of the GDA-J/F3 antigen with the sensitivity of its epitopes to denaturing and non-denaturing detergents impedes the purification and a more detailed biochemical characterization of this basement membrane protein.

Like all the components of the skin basement membrane zone, the GDA-J/F3 antigen is likely to play a role in dermal-epidermal adhesion. Simultaneously, it is a candidate protein/gen for a group of genetic blistering disorders of the skin, the dystrophic EB. In many patients with this disorder, mutations in the collagen VII gene have been defined that lead to absence or structural alterations and impaired function of the anchoring fibrils (14). However, in a number of EBD families the search for mutations has not revealed a defect in the collagen VII gene, suggesting the presence of other causative abnormalities (48). For analyses of such abnormalities, clarification of the protein and gene structure of the GDA-J/F3 antigen is pivotal.

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