Type XVI Collagen is Expressed in Factor XIIIa$^+$ Monocyte-Derived Dermal Dendrocytes and Constitutes a Potential Substrate for Factor XIIIa

Atsushi Akagi, Shingo Tajima, Akira Ishibashi, Yuko Matsubara,* Makoto Takehana,* Shizuko Kobayashi,* and Noriko Yamaguchi†

Department of Dermatology, National Defense Medical College, Saitama, Japan; *Department of Physiology and Anatomy, Kyoritsu College of Pharmacy, Tokyo, Japan; †Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

We have previously reported that connective tissue cells in the superficial dermis preferentially express α_1(XVI) collagen rather than those in the lower dermis. Double immunofluorescence labeling using the antibodies for α_1(XVI) collagen and factor XIIIa (plasma transglutaminase), which is a marker of dermal dendrocytes, demonstrated that both antibodies reacted with the same cells in the superficial dermis of normal skin as well as the lesional skins of dermal dendrocyte-related disorders, dermatofibroma, and psoriasis. Dermal dendrocytes are considered to be established by a culture of peripheral blood monocytes in the presence of granulocyte macrophage-colony stimulating factor and interleukin-4. Reverse transcription–polymerase chain reaction, metabolic labeling, and immunofluorescence studies demonstrated that treatment of CD14$^+$ peripheral blood monocytes with granulocyte macrophage-colony stimulating factor/interleukin-4 over a period of 8 d resulted in the induction of α_1(XVI) collagen as well as factor XIIIa. The physiologic significance of colocalization of α_1(XVI) collagen and factor XIIIa in the tissue and their coordinate induction in CD14$^+$ monocyte-derived dendritic cells *in vitro* was studied. Considerable incorporation of [^{3}H]putrescine by factor XIIIa into recombinant noncollagenous domain (NC) 11 but not into collagenous domain (COL) 1·NC1 domain of the α_1(XVI) polypeptide was found. Incubation of recombinant NC11 of α_1(XVI) polypeptide with factor XIIIa *in vitro* produced a covalent cross-linking complex on sodium dodecylsulfate–polyacrylamide gel electrophoresis. The results indicate that α_1(XVI) collagen is constitutively expressed by most dermal dendrocytes in the skin and dendritic cells differentiated from peripheral blood monocytes *in vitro*. Type XVI collagen is expressed in factor XIIIa$^+$ dermal dendrocytes and may form an intermolecular cross-linking through NC11 domain by the reaction catalyzed by factor XIIIa contributing to the structural integrity of factor XIIIa$^+$ dendritic cell-rich tissues.

Key words: collagen XVI/cross-linking/dendrocyte/factor XIIIa/monocyte. J Invest Dermatol 118:267–274, 2002

On the basis of structure and function, collagens are generally divided into fibril-forming collagens and nonfibril-forming collagens. Unlike fibril-forming collagens, nonfibril-forming collagens are very heterogeneous in size and have imperfections in the GLy–X–Y repeating sequence as a common feature. In the latter class, type IX, XII, and XIV collagens form a subgroup named the fibril-associated collagens with interrupted triple helices (FACIT) (Vuorio and Crombrugghe, 1990; Fukai *et al*, 1994). Type XVI collagen polypeptide designated as α_1(XVI) consists of 10 collagenous domains, which were interspersed with 11 noncollagenous domains exhibiting several structural features characteristically seen in members of FACIT (Pan *et al*, 1992; Yamaguchi *et al*, 1992). It has been shown that the FACIT members are localized on the surface of major collagen fibrils and may serve as molecular bridges that are responsible for maintaining the structural integrity of the extracellular matrices. Although the structural similarities between FACIT and type XVI collagen may suggest similar function, no information on its physiologic function is available. Type XVI collagen in the adult mouse has been demonstrated to distribute widely in the heart, kidney, intestine, ovary, testis, eye, arterial wall, and smooth muscle (Lai and Chu, 1996), supporting the fact that collagen may contribute to the structural integrity of various tissues. In the skin, it has been demonstrated that specific type XVI collagen gene transcripts and its biosynthesis are detected in cultured dermal fibroblasts and keratinocytes, and its expression is closely related to cell growth arrest (Grassel *et al*, 1996, 1998; Tajima *et al*, 2000). By an immunohistochemical study, we and colleagues have previously demonstrated that the antibody against α_1(XVI) chain reacted preferentially with the fixed connective tissue cells and matrix in the papillary dermis rather than those in the reticular dermis (Akagi *et al*, 1999; Grassel *et al*, 1999), suggesting that type XVI collagen-positive cells in the papillary dermis may represent a specific phenotype of the connective tissue cells.

Manuscript received April 29, 2001; revised October 11, 2001; accepted for publication October 25, 2001.

Reprint requests to: Dr. Shingo Tajima, Department of Dermatology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. Email: tajimas@ndmc.ac.jp

Abbreviations: DD, dermal dendrocytes; FACIT, fibril-associated collagens with interrupted triple helices.

Dendritic cells are defined as a group of cells that are bone marrow-derived, express major histocompatibility complex class II antigens, show little or no endocytotic or phagocytic activity, and display potent accessory function in antigen presentation. Cutaneous cells with dendritic morphology include cells that reside primarily in the epidermal layer, such as Langerhans cells and poorly defined cells of similar contour in the underlying dermis [dermal dendrocytes (DD)] (Headington, 1986; Hoyo *et al*, 1993). DD express several monocyte/macrophage markers. These features along with the expression of major histocompatibility complex class II antigens have promoted the speculation that DD represent a subset of dendritic, antigen-presenting macrophages (Nickoloff, 1991). It has been demonstrated that the treatment of $CD14^+$ peripheral blood monocytes with granulocyte/macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4 induces differentiation into dendritic cells (DD), which express coagulation factor XIIIa (FXIIIa; plasma transglutaminase) (Young *et al*, 1990; Sallusto and Lanzavecchia, 1994). On the other hand, it has been reported that DD arise from embryonic mesenchymal cells and represent a population of resident cells of the dermis identified by virtue of the immunohistochemical expression of FXIIIa (Gibran *et al*, 1996). FXIIIa-positive cells are present preferentially in the papillary dermis around the superficial blood vessels in normal skin (Cerio *et al*, 1989a; Nestle *et al*, 1993). Although the question whether DD represent a subset of dendritic monocytes or unique skin resident cells with distinctive characteristics remains to be answered, these cells appear to be of critical importance in cutaneous pathophysiology. For example, increased number or size of DD has been identified in various skin disorders: psoriasis and atopy (Cerio *et al*, 1989a), Kaposi's sarcoma associated with the acquired immune deficiency syndrome (Nickoloff and Griffiths, 1989), dermatofibroma (Cerio *et al*, 1989b), acute graft versus host disease (Yoo *et al*, 1998), morphea, and scars (Gilmour *et al*, 2000).

Coagulation FXIII is a protransglutaminase involved in the final stage of the coagulation pathway. FXIII is a tetrameric protein consisting of two pairs of subunits (two a and two b) with the enzyme activity present in subunit a (FXIIIa) (Muszbek *et al*, 1996; Aeschlimann and Thomazy, 2000). It has been shown that FXIIIa stabilizes clot formation by cross-linking fibrin, fibronectin to collagen types I, II, III, and V, or von Willebrand factor to collagen (Mosher *et al*, 1980; Mosher, 1984; Bockenstedt *et al*, 1986). There have been no previous reports describing collagen–collagen cross-linking of collagenous protein itself by FXIIIa.

The restricted localization of the FXIIIa-positive and α_1(XVI) collagen-positive cells in the superficial dermis suggests that they share a common phenotype of the connective tissue cells in the papillary dermis. This led us to study immunohistochemically normal skin as well as DD-related skin diseases using both anti-FXIIIa and anti-type XVI collagen antibodies. In addition, based upon the demonstration that DD are induced to differentiate from cultured $CD14^+$ peripheral blood monocytes, we tried to detect α_1(XVI) mRNA or its polypeptide synthesis in the cultured peripheral blood monocytes treated with GM-CSF and IL-4. Finally, we attempted to study whether type XVI collagen is a potent substrate for FXIIIa -mediated cross-linking.

MATERIALS AND METHODS

Tissue samples Skin samples were obtained from normal volunteers (n = 4) (ages; 28–35, male/female; 4:0) or the patients with dermatofibroma (n = 4) (ages; 21–40, male/female; 3:1), or psoriasis vulgaris (n = 4) (ages; 41–54, male/female; 4:0). Diagnosis of dermatofibroma and psoriasis was made by typical histologic features.

Double immunofluorescence labeling of the skin or cultured peripheral blood monocytes The skin specimens were embedded in optimal cutting temperature (OCT) compound (Sakura Finetechnical, Japan) and snap-frozen. The specimens were cut into 5 μm sections and fixed with acetone. $CD14^+$ peripheral blood monocytes (See below) were cultured on the slide glass (Becton Dickinson Labware, Franklin Lakes, NJ) and fixed with acetone. The samples were incubated with both rabbit affinity-purified anti-α_1(XVI) collagen antibody raised against synthetic peptide TQPTRRVFPRGLPEEFAL in the N-terminal noncollagenous domain (NC11) of α_1(XVI) polypeptide (Akagi *et al*, 1999) and sheep anti-human FXIIIa antibody (Enzyme Research Laboratories, South Bend, IN) for 24 h at 1:500 dilution for anti-α_1(XVI) collagen antibody or 1:100 dilution for anti-FXIIIa antibody. The sections or cultured cells were incubated with rhodamine-conjugated swine anti-rabbit IgG (Dako, Glostrup, Denmark) at 1:40 dilution and fluorescein isothiocyanate-conjugated donkey anti-sheep IgG (Chemicom, Temecula, CA) at 1:50 dilution for 2 h, then washed with phosphate-buffered saline exhaustively. Evaluation of fluorescence was performed with a confocal laser scan microscope (LSM410, Carl Zeiss, Jena, Germany). The number of $\alpha_1(XVI)^+$ or $FXIIIa^+$ cells in the skin specimens or cultured peripheral blood monocytes was calculated from the observation of 50 cells in a random, nonoverlapping field under a microscope. Values were obtained from four different skin samples or four different culture dishes. Statistical study was performed using one-sided Student's t test and statistical significance at $p < 0.01$ was considered to be significant.

Culture of peripheral blood monocytes Peripheral blood was obtained from normal donors. Peripheral blood mononuclear cells were isolated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Peripheral blood mononuclear cells (1×10^8) were incubated with 200 μl of microbeads conjugated with monoclonal antihuman CD14 antibody in 800 μl of MACS buffer [phosphate-buffered saline/2 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0/ 0.5% bovine serum albumin (BSA)] at 4°C for 30 min. The cells immobilized with CD14 microbeads were separated by a magnetic cell separator (MidiMACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's protocol. More than 90% purity of $CD14^+$ cells was routinely obtained. The $CD14^+$ cells were plated in six-well or 10 cm diameter tissue culture plates (Becton Dickinson Labware) at a density of 2×10^6 cells per plate in 3 ml or 10 ml of RPMI medium containing 10% fetal bovine serum and incubated at 37°C for 4 h in a humidified atmosphere and CO_2. After aspiration of nonadherent cells, adherent cells were treated for 0, 4, and 8 d with recombinant GM-CSF (10 ng per ml) (Genzyme, Cambridge, MA) and recombinant IL-4 (10 ng per ml) (Genzyme) in RPMI medium containing 10% fetal bovine serum (Young *et al*, 1990; Sallusto and Lanzavecchia, 1994).

Reverse transcription–polymerase chain reaction (RT–PCR) assay Total cellular RNA was isolated as described previously (Chomczynski and Sacchi, 1987). Ten micrograms of total RNA was treated with 1 unit of DNase I (Ambion, Austin, TX**)** for 15 min and inactivated with EDTA. RT–PCR was performed with a 5′ primer [5′ CTGTGGCTGTAGCTGACCTC3′ (nucleotide number 123–142)] and a 3′ primer [5′ AGATGCAGGACACAAAGTCG3′ (nucleotide number 606–625)] based on the sequence of NC11 domain of human α_1(XVI) cDNA (Pan *et al*, 1992), or with a 5′ primer [5′GGGAAG-CTTTCAGAAACTTCCAGGACCGCCTTTGG3′ (nucleotide number 88–109)] and a 3′ primer [5′GGAATTCCTGGATGGACAGCCGCA-CAGACCTGTC3′ (nucleotide number 502–526)] corresponding to the 5′ end sequence of human FXIIIa cDNA (Grundmann *et al*, 1986). The first strand cDNA was synthesized using total RNA (1 μg per assay), a 3′ primer and reverse transcriptase at 42°C for 1 h. The reaction was run for 35 cycles with denaturation at 94°C for 1 min, annealing at 62°C for 2 min, and extension at 72°C for 5 min in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT) (Kawasaki, 1990). The quality of the cDNA synthesis was monitored by amplifying the human glyceraldehyde-3-phosphate-dehydrogenase gene. The primer sequences of glyceraldehyde-3-phosphate-dehydrogenase were: 5′ primer (5′GAA-GGTGAAGGTCGGAGTC3′) and 3′ primer (5′GAAGATGGTGAT-GGGATTTC3′) (Maxim Biotech, San Francisco, CA). Amplification for glyceraldehyde-3-phosphate-dehydrogenase was carried out with 28 cycles of 1 min denaturation at 94°C, 1 min annealing at 62°C, and 1 min extension at 72°C. The reverse transcription–PCR products were analyzed by an electrophoresis on 1% agarose. DNA fragments amplified by PCR were extracted from agarose gel and ligated into the plasmid vector (pCRII) (Invitrogen, Carlsbad, CA). The constructs were transformed into an *Escherichia coli* strain JM109 (Invitrogen). The plasmid DNA was purified with Wizard plus minipreps (Promega, Madison, MI) and sequenced by the dideoxy chain termination method using a Sequenase kit (Sanger *et al*, 1977). The nucleotide sequences were found to be identical to those previously reported (Grundmann *et al*, 1986; Pan *et al*, 1992).

Biosynthesis of α_1(XVI) collagen $CD14^+$ cultured monocytes that had been treated with or without GM-CSF/IL-4 for 8 d were labeled with [^{35}S]methionine (40 TBq per mmol; Amersham Pharmacia Biotech, Buckinghamshire, U.K.) for 24 h in RPMI medium deficient in fetal bovine serum and L-methionine supplemented with 30 μg per ml ascorbic acid. For comparison, fibroblasts obtained from fetus skin established from a routine explant method were labeled with [^{35}S]methionine as well. Culture medium was harvested and mixed with protease inhibitor cocktails (1 mM EDTA, 1 mM N-ethylmaleimide, and 1 mM phenylmethylsulfonyl fluoride), then stored at –80°C until use. Cells were homogenized in 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 1 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, and 0.3% nonidet P-40 at 4°C. The supernatant of cell pellet and culture medium was individually applied to the anti-α_1(XVI) NC11 antibody column (Akagi *et al*, 1999). Bound proteins were eluted with 0.1 M citrate buffer at pH 6.0, pH 5.0, pH 4.0, and pH 3.0. The elution of the proteins was monitored by liquid scintillation spectrometer (Beckman LS 9800, Beckman Coulter, Fullerton, CA). Preliminary experiments showed that most of the radioactivity (more than 92%) was eluted at pH 3.0. The fraction of pH 3.0 was lyophilized and subjected to 5–20% gradient sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The gels were fixed in methanol/acetic acid, dried *in vacuo*, then subjected to autoradiography for 48 h at –80°C.

Western blot assay of FXIIIa in cultured monocytes $CD14^+$ peripheral blood monocytes treated without or with GM-CSF/IL-4 for 8 d were washed with phosphate-buffered saline three times, then further cultured in serum-free RPMI medium for 24 h. Conditioned medium was mixed with protease inhibitor cocktails, then the proteins were precipitated with 50% ammonium sulfate (313 mg per ml). Cells were trypsinized and suspended in 10 mM Tris–HCl, pH 7.4, 1 mM EDTA, then disrupted by sonication (Handy Sonic UR-20P, Tomy Seiko Co., Tokyo, Japan) with five 30 s bursts at 4°C. Insoluble materials were pelleted by centrifugation at 10,000 × *g* for 20 min and the proteins in the supernatant were precipitated with 10% trichloroacetic acid. The pellets in the medium and cells were dissolved in 0.1 M Tris–HCl, pH 8.5 containing 1% SDS, boiled at 90°C for 2 min, then resolved on a 5–20% gradient SDS–PAGE in the reducing condition. Protein content in the medium and cell lysate was determined with the protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard, and the concentration of the sample was adjusted to be equivalent in each well of SDS–PAGE. The proteins isolated from culture medium and cells of established human skin fibroblasts, as well as FXIIIa generated by the reaction of FXIII and thrombin, and tissue transglutaminase prepared from liver (Sigma, St Louis, MO) were also subjected to 5–20% gradient SDS–PAGE under a reducing condition. The samples were electrophoretically transferred from gels to nitrocellulose membrane (Schleicher & Schuell, Keene, NH) (Towbin *et al*, 1979). The membranes were incubated with anti-FXIIIa antibody at 1:1000 dilution for 1 h, and then reacted with anti-sheep IgG at 1:2000 dilution for 1 h. Antigen–antibody was visualized by a chemiluminescence kit (Amersham Pharmacia Biotech).

Preparation of recombinant COL1·NC1 and NC11 of α_1 (XVI) molecule For the expression of the amino terminal flag tagged COL1·NC1 (amino acids 1472–1603) and NC11 (amino acids 22–333) domains of α_1(XVI) collagen, DNA fragments were amplified by PCR using the cDNA clones NYh-4 (Yamaguchi *et al*, 1992) and NYh-6 isolated from human heart cDNA library (Clontech, Palo Alto, CA) (unpublished data) as templates and primers with a *Nhe*I and *Not*I restriction site at the ends. The following primers were chosen for each of the two recombinant peptides of α_1(XVI) collagen: flag tagged COL1·NC1 fragment, 5′ primer [5′ AGCGCTAGCTGACTACAAGGACGACGATGACAAGGGACGACCAGGGCCTCCAGGGAAG3′ (nucleotide number 193–240)] and 3′ primer [5′GTCGGCGGCCGCGAATTTCAGCCAAAAGGCCCCT3′ (nucleotide number 1130–1152)]; flag tagged NC11 fragment, 5′ primer [5′AGCGCTAGCTGACTACAAGGACGACGATGACAAGGCAAATACAGGTGCACAATGCCCA3′ (nucleotide number 4567–4590)] and 3′ primer [5′GTCGGCGGCCGCGGTGGCTAAGAGGGAGCAAGTGTGACATTGC3′ (nucleotide number 4949–4970)]. We sequenced the entire PCR products and found an identical sequence to the previously published one. The PCR products were cloned into the modified episomal expression vector pCEP-Pu (Kofeldt *et al*, 1997). Human embryonic kidney cells (293-EBNA) were purchased from Invitrogen and used for transfection. Stably transfected cells were selected by 0.5 μg per ml of puromycin, and serum-free conditioned medium was collected (Yamaguchi *et al*, 1999). Conditioned medium was dialyzed against 50 mM Tris–HCl, pH 7.5, 0.15 M NaCl and passed over a column immobilized with anti-FLAG antibody (Sigma). Bound proteins were eluted with 0.1 M glycine–HCl, pH 3.5 and neutralized immediately after elution. Purified COL1·NC1-Nflag and NC11-Nflag were dialyzed against phosphate-buffered saline. Purity of recombinant proteins was evaluated by SDS–PAGE. Identification of recombinant α_1(XVI) NC11 polypeptide was performed by western blot analysis using anti-α_1(XVI) antibody raised against synthetic peptides of the NC11 domain (Akagi *et al*, 1999) and partial N-terminal amino acid sequence using a protein sequencer (Beckman LF3000).

Incorporation of [^{3}H]putrescine by FXIIIa into collagenous and noncollagenous proteins Pepsin-solubilized human skin collagens type I and III (Fuji Pharmaceutical, Toyama, Japan) dissolved in 50 mM Tris–HCl pH 7.4, 0.15 M NaCl were heat denatured at 60°C for 1 h to produce α-chains. Plasma fibronectin was purchased from Life Technology (Grand Island, NY). FXIII prepared from human plasma was purchased from Cortex Biochem (San Leandro, CA). *N,N′*-dimethylcasein, BSA and thrombin were obtained from Sigma. Incorporation of [1,4-^{3}H]putrescine (1.0 TBq per mmol; Amersham) by FXIIIa into various collagenous and noncollagenous polypeptides was determined according to the method of Bowness *et al* (1987). Activation of FXIIIa was performed by incubating 1 μg of FXIII per ml with 0.1 μg thrombin per ml at 37°C for 15 min in 0.1 M Tris–HCl, pH 8.5, 5 mM $CaCl_2$ in a total volume of 40 μl. One microliter of FXIIIa solution was added to the reaction mixture consisting of [^{3}H]putrescine (7 μCi/1.8 μM), protein substrates (750 nM) in the buffer (0.1 M Tris–HCl, pH 8.5 containing 5 mM $CaCl_2$ and 1 mM dithiothreitol) and incubated at 37°C for 0, 30, 60, and 120 min. An enzyme blank containing all reagents with excess EDTA (50 mM) was run with each set of tubes. FXIIIa was used as a control substrate to measure the autocatalytic reaction of FXIII itself. After incubation, the proteins were precipitated with 10% trichloroacetic acid at 4°C. The precipitates were washed with 10% trichloroacetic acid four times, then with ethanol/ether (1:1) once. The proteins in the precipitates were dissolved with 1% SDS by boiling for 2 min, and the radioactivities were counted with liquid scintillation spectrometer (Beckman LS 9800). Three individual assays were performed in duplicate.

Cross-linking formation of recombinant NC11 domain of α_1(XVI) polypeptide by FXIIIa Activation of FXIIIa was performed as described above. NC11 fragment (1 μg), plasma fibronectin (1 μg) or type III collagen (Fuji Pharmaceutical) 1 μg was incubated with FXIIIa solution at 37°C for 60 min in 0.1 M Tris–HCl, pH 8.5, 5 mM $CaCl_2$ in a total volume of 20 μl. Control experiments were set up by deletion of thrombin, $CaCl_2$, or α_1(XVI) NC11. The reaction products were denatured by heating at 100°C for 1 min and resolved on 10–20% gradient SDS–PAGE under a reducing condition, then blotted on to the membranes. The membrane was incubated with anti-α_1(XVI) NC11 rabbit antibody at 1:1000 dilution, with anti-fibronectin rabbit antibody (Dako) at 1:2000 dilution or with anti-type III collagen mouse antibody (Fuji Pharmaceutical) at 1:1500 dilution for 1 h, then reacted with peroxidase conjugated secondary antibody at 1:2000 dilution for 1 h. Antigen–antibody complex was detected by chemiluminescence (Amersham Pharmacia Biotech).

RESULTS

Localization of α_1(XVI)$^+$ and FXIIIa$^+$ cells in the dermis In normal skin, anti-FXIIIa antibody reacted with the cells with a dendritic morphology in the upper dermis as has been previously reported (Cerio *et al*, 1989a; Nickoloff and Griffiths, 1989; Nestle *et al*, 1993; Gilmour *et al*, 2000) (**Fig 1*a***). Anti-α_1(XVI) collagen antibody recognized the majority of FXIIIa-positive cells (**Fig 1*b***), but the antibody also recognized a few FXIIIa-negative cells in the superficial dermis (*arrow* in **Fig 1*b***). There was a negligible population of α_1(XVI)$^-$/FXIIIa$^+$ in the superficial dermis. The antibody did not react with epidermal Langerhans cells (not shown) but reacted weakly with epidermis, which is consistent with a previous study (Grassel *et al*, 1999). As dermatofibroma is considered to have originated from FXIIIa$^+$ DD and is referred to as dermal dendrocytoma (Cerio *et al*, 1989b), skin specimens from dermatofibroma were subjected to immunohistochemical analysis with anti-α_1(XVI) antibody. In the lesional skin of dermatofibroma, majority of tumor cells were positive with both anti-α_1(XVI) collagen and anti-FXIIIa antibodies (**Fig 1*c*, *d***). A

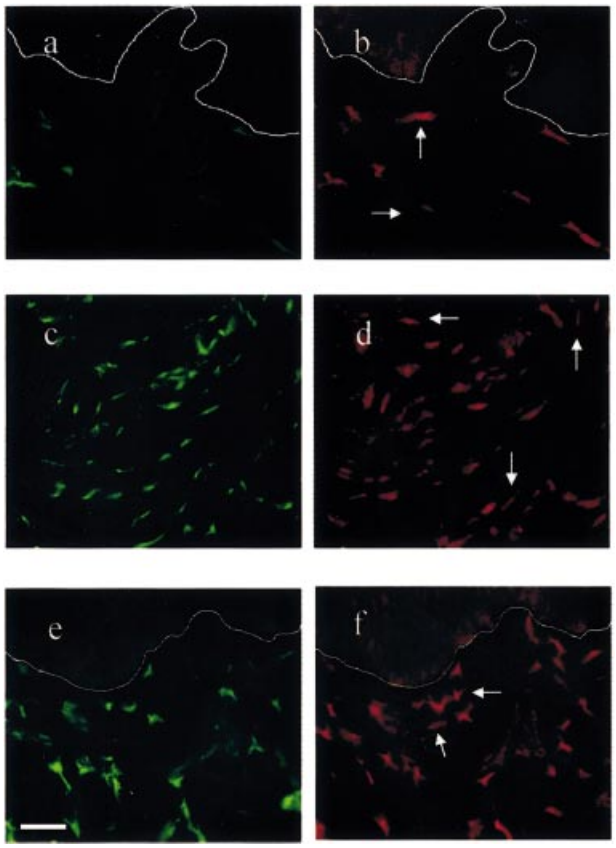

Figure 1. Double immunofluorescence of normal skin and lesional skins of dermatofibroma and psoriasis with anti-α_1(XVI) collagen and anti-FXIIIa antibodies. Cryostat sections of the skin specimens taken from normal skin (*a*, *b*), dermatofibroma (*c*, *d*), and psoriasis (*e*, *f*) were incubated with the antibodies [rabbit anti-α_1(XVI) NC11 collagen antibody at 1:500 dilution and sheep anti-FXIIIa antibody at 1:100 dilution] for 24 h at 4°C. The sections were incubated with fluorescein isothiocyanate-conjugated anti-sheep IgG and rhodamine-conjugated anti-rabbit IgG for 2 h. Evaluation of fluorescence was performed with a confocal laser scan microscope. (*a*, *c*, *e*) Anti-FXIIIa antibody. (*b*, *d*, *f*) Anti-α_1(XVI) NC11 collagen antibody. Solid line in *a*, *b*, *e*, *f* indicates dermoepidermal junction. Dermoepidermal junction was identified by anti-type IV collagen antibody. Original magnification, ×200. $\alpha_1(XVI)^+/FXIIIa^-$ population was indicated by *arrows* (*b*, *d*, *f*). *Scale bar*: 50 μm.

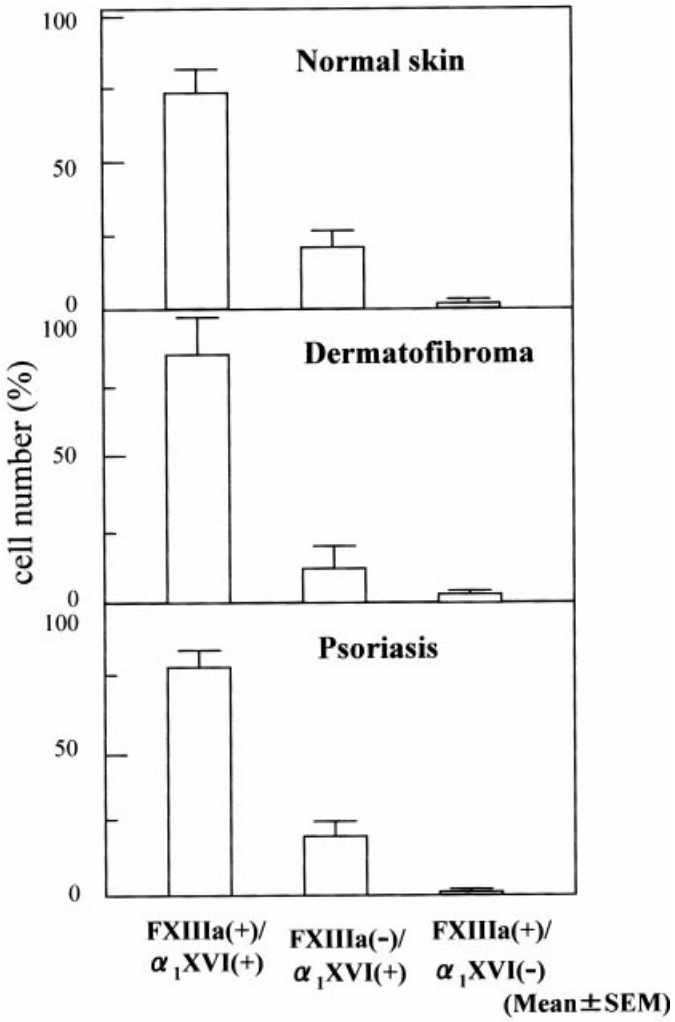

Figure 2. Determination of α_1(XVI)-positive or FXIIIa-positive cells in normal skin, dermatofibroma, and psoriatic skin. Skin specimens taken from normal individual (*upper panel*), dermatofibroma (*middle panel*), and psoriatic patient (*lower panel*) were immunoreacted with both anti-α_1(XVI) NC11 collagen and anti-FXIIIa antibodies. The number of positive cells was counted from the observation of 50 cells in random, nonoverlapping fields of four different samples. Values indicate mean ± SEM.

few $\alpha_1(XVI)^+/FXIIIa^-$ cells were found (*arrows* in **Fig 1*d***). There were no $\alpha_1(XVI)^-/FXIIIa^+$ cells. In psoriatic skin, the increase in the number of $FXIIIa^+$ DD has been demonstrated (Cerio *et al*, 1989a). In lesional skin of psoriasis, similar results to those of normal skin were obtained except for the relatively abundant number of $FXIIIa^+$ or $\alpha_1(XVI)^+$ cells (**Fig 1*e*, *f***). Determination of the number of cells positive for either anti-α_1(XVI) collagen or anti-FXIIIa antibody in normal skin showed that the populations of $\alpha_1(XVI)^+/FXIIIa^+$ were predominant and comprise 71 ± 5%, whereas $\alpha_1(XVI)^+/FXIIIa^-$ and $\alpha_1(XVI)^-/FXIIIa^+$ comprise 23 ± 3% and less than 2 ± 1%, respectively. The cell populations in dermatofibroma and psoriatic skin were similar to those of normal skin (**Fig 2**).

Induction of α_1(XVI) and FXIIIa in $CD14^+$ monocyte-derived dendritic cells To confirm that the majority of DD in the dermis consist of $\alpha_1(XVI)^+FXIIIa^-$cells, an *in vitro* culture system was employed, which induces the differentiation of peripheral blood monocytes into dendritic cells. RT–PCR using α_1(XVI) NC11-specific primer pairs showed a very faint DNA fragment with 493 bp in the adherent cells prior to treatment (**Fig 3*a***, *lane 0*). Treatment of adherent $CD14^+$ peripheral blood monocytes with GM-CSF/IL-4 for 8 d relatively increased 493 bp DNA fragment (**Fig 3*a***, *lane 8*). The induction appeared to be dependent on the period of the treatment (**Fig 3*a***). The intensity of DNA fragment with 441 bp produced by RT–PCR using FXIIIa-specific primers was also relatively increased time-dependently after 8 d treatment (**Fig 3*b***). A low degree of FXIIIa mRNA was detectable by RT–PCR in the cells before the treatment with GM-CSF/IL-4 (**Fig 3*b***, *lane 0*), which is consistent with previous reports that some extent of FXIIIa is expressed in circulating peripheral blood monocytes (Henriksson *et al*, 1985; Weisberg *et al*, 1987). FXIIIa mRNA was not detected in the cultured skin fibroblasts (**Fig 3*b***, *lane F*).

Immunoaffinity assay using anti-α_1(XVI) NC11 antibody column showed four distinct polypeptides with the molecular weights of 220, 180, 130, and 62 kDa in both medium and cell layer fractions in the treated monocytes, whereas no immunoreactive polypeptide was detected in the untreated (control) monocytes. In dermal fibroblasts, four immunoreactive polypeptides with the same molecular sizes were detected (**Fig 4*a***). We considered 220 kDa polypeptide as an intact α_1(XVI) collagen chain, and 180 (Grassel *et al*, 1996), 130, and 62 kDa polypeptides as proteolytically processed α_1(XVI) chains.

To know whether FXIIIa is induced in the dendritic cells differentiated from monocytes, the proteins prepared from the medium and cell lysate of cultured monocytes were analyzed by western blot analysis using anti-FXIIIa antibody. Western blot demonstrated that anti-FXIIIa antibody specifically reacted with authentic FXIIIa with a molecular weight of 82 kDa, but did not cross-react with tissue transglutaminase. FXIIIa levels in both culture medium and cell lysate were extremely enhanced in the treated monocytes compared with the untreated monocytes. No detectable FXIIIa level was found in the medium and cells of cultured fibroblasts (**Fig 4*b***).

Treatment of adherent $CD14^+$ peripheral blood monocytes with GM-CSF/IL-4 resulted in a change of cell shape. Immunofluorescence labeling of cultured $CD14^+$ monocytes demonstrated that the antibodies for α_1(XVI) collagen and FXIIIa reacted with identical cells. The number of $\alpha_1(XVI)^+FXIIIa^-$cells was very few before the treatment (1.8–2.5% of total cells), but markedly increased (97–99% of total cells) after treatment for 8 d (**Fig 5**).

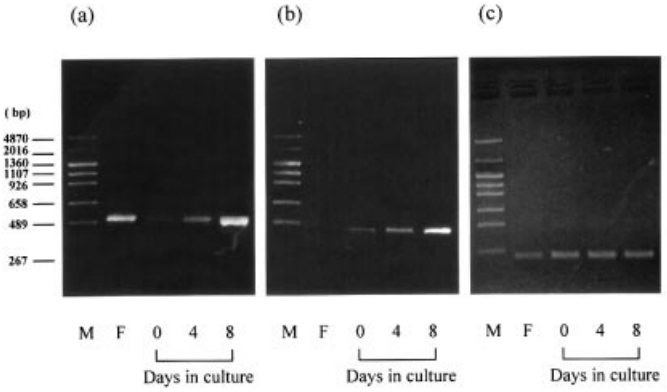

Figure 3. Expression of α_1(XVI) and FXIIIa in cultured peripheral blood monocytes treated with GM-CSF/IL-4. $CD14^+$ peripheral blood monocytes were isolated, then plated in tissue culture plate. Adherent cells were received the treatment of GM-CSF (10 ng per ml) and IL-4 (10 ng per ml) for 0, 4, and 8 d. Control cells (treatment for 0 d) indicate adherent cells after 4 h of inoculation. Total cytoplasmic RNA was isolated from the cells, and 10 μg of total RNA was treated with DNase I for 15 min. Reverse transcription–PCR was performed using (*a*) α_1(XVI) collagen, (*b*) FXIIIa or (*c*) glyceraldehyde-3-phosphate-dehydrogenase specific oligomers as primers. PCR products were resolved by electrophoresis on 1% agarose gel. *Lane F* indicates positive control assay using the RNA extracted from cultured normal skin fibroblasts. *Lane M* indicates molecular markers.

Recombinant NC11 domain of α_1(XVI) is a potential substrate for FXIIIa The localization of $FXIIIa^+$ and $\alpha_1(XVI)^+$ cells in the superficial connective tissue cells *in vivo* and

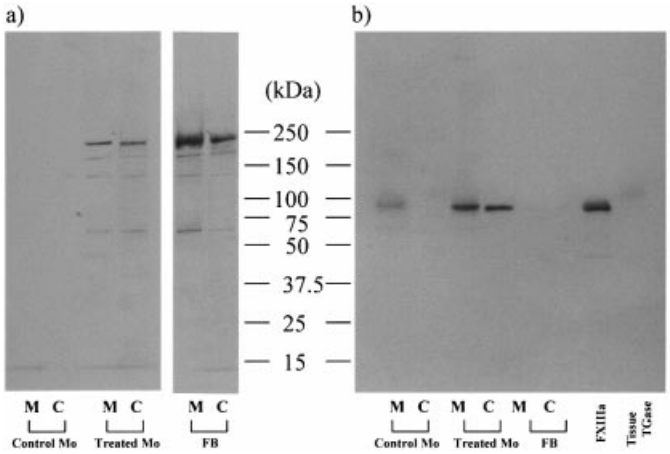

Figure 4. Biosynthesis of α_1(XVI) collagen and FXIIIa in the dendritic cells differentiated from peripheral blood monocytes. $CD14^+$ peripheral blood monocytes were plated and treated with GM-CSF (10 ng per ml) and IL-4 (10 ng per ml) for 0 and 8 d. (*a*) Cells were labeled with [^{35}S]methionine for the final 24 h (Treated Mo). For control experiments (treatment for 0 d), adherent monocytes were labeled for 24 h in the absence of GM-CSF and IL-4 (Control Mo). For comparison, cultured normal skin fibroblasts were grown to confluent density, then labeled with [^{35}S]methionine for 24 h (FB). Culture medium and cells were harvested, then the proteins were adsorbed with anti-α_1(XVI) NC11 antibody column and resolved on SDS–PAGE under a reducing condition. The gels were processed to autoradiography. (*b*) Cells before (Control Mo) or after the treatment (Treated Mo) with GM-CSF/IL-4 were incubated with serum-free medium for 24 h. Proteins in the conditioned medium and cell lysate harvested from treated and untreated monocytes were resolved on SDS–PAGE under a reducing condition, blotted on to the filters, then incubated with anti-FXIIIa antibody. For comparison, conditioned medium and cell lysate of cultured skin fibroblasts were also subjected for analysis (FB). Authentic FXIIIa and tissue transglutaminase were subjected to western blot analysis as positive and negative controls, respectively. M, medium fraction; C, cell fraction.

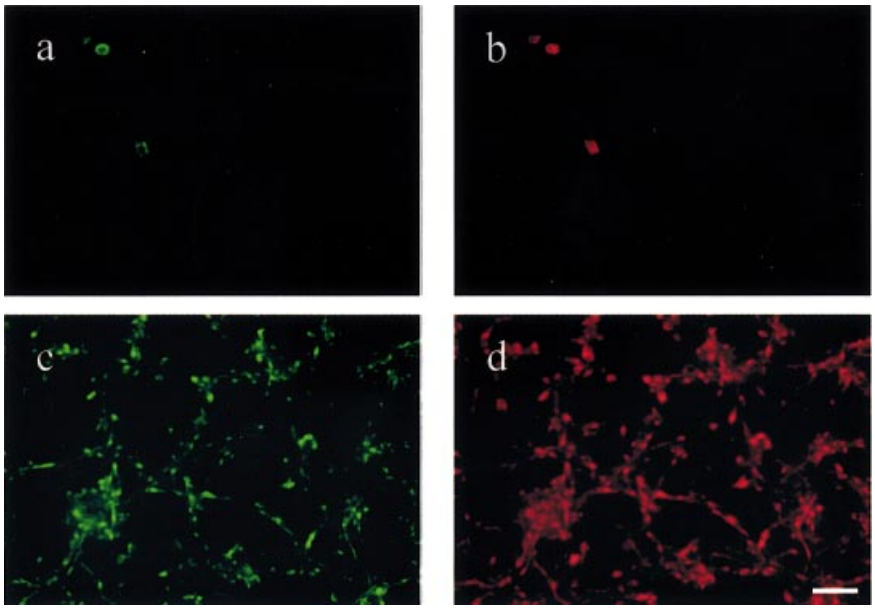

Figure 5. Immunostaining of cultured peripheral blood monocytes with anti-α_1(XVI) collagen and anti-FXIIIa antibodies. $CD14^+$ peripheral blood monocytes were plated and treated with GM-CSF (10 ng per ml) and IL-4 (10 ng per ml) for 0 and 8 d. Cells were fixed with acetone, then incubated with anti-FXIIIa (*a*, *c*) and anti-α_1(XVI) collagen (*b*, *d*) antibodies. (*a*, *b*) Immunostaining prior to treatment with GM-CSF/IL-4; (*c*, *d*) Immunostaining after treatment with GM-CSF/IL-4. Original magnification ×120. *Scale bar*: 50 μm.

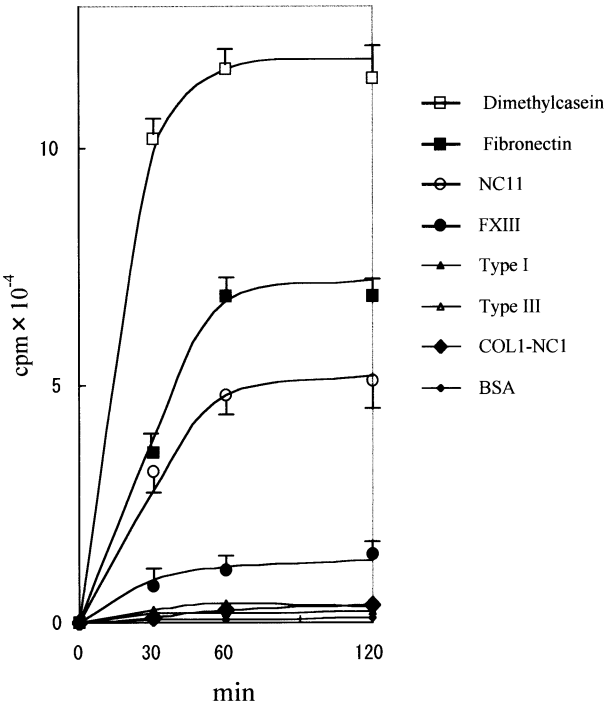

Figure 6. Factor XIIIa-catalyzed [^{3}H]putrescine incorporation in collagenous and noncollagenous proteins. [^{3}H]putrescine (7 μCi) and 750 nM of each proteins, *N,N′*-dimethylcasein, fibronectin, α_1(XVI) NC11, FXIII, type I collagen, type III collagen, α_1(XVI) COL1·NC1 and BSA were incubated at 37°C for 0, 30, 60, and 120 min in the presence of FXIIIa. Incorporation of [^{3}H]putrescine was measured by trichloroacetic acid precipitation. The net incorporation was calculated by subtraction of the value in the presence of excess EDTA. Values indicate mean ± SEM.

coordinate expression of α_1(XVI) and FXIIIa in the monocyte-derived dendritic cells led us to investigate whether the α_1(XVI) chain can be a potential substrate for FXIIIa. We determined the incorporation of [^{3}H]putrescine by FXIIIa into two different domains (COL1·NC1 and NC11) of the α_1(XVI) polypeptide with BSA and type I and type III collagens as negative controls (Bowness *et al*, 1987), and two well-characterized transglutaminase substrates *N,N′*-dimethylcasein and plasma fibronectin as positive controls (Fesus *et al*, 1986; Bowness *et al*, 1987; Aeschlimann and Paulsson, 1991). Incubation of COL1·NC1 domain of α_1(XVI) with FXIIIa gave only background levels of incorporation, comparable with the reaction with BSA and type I and type III collagens. In contrast, the NC11 domain of α_1(XVI) showed a significant incorporation of [^{3}H]putrescine to the extent comparable with fibronectin, but to a lesser extent than dimethylcasein. A low degree of autocatalytic reaction of FXIII was found (**Fig 6**).

To investigate further whether FXIIIa actually mediates covalent cross-linking of the α_1(XVI)NC11 domain, this domain was incubated with FXIIIa, and reaction products were analyzed by SDS–PAGE under a reducing condition. We used fibronectin as a positive control and type III collagen as a negative control. Western blot analysis using anti-α_1(XVI) NC11 antibody showed that α_1(XVI) NC11 formed high molecular complexes in addition to the intact α_1(XVI) NC11 domain (35 kDa). The apparent molecular weight of the complexes ascertained by SDS–PAGE were 72 kDa and $> 1 \times 10^6$ kDa (at the top of the gel) (**Fig 7*a***, *lane 1*). These complexes were not found when the incubation mixture lacked thrombin (**Fig 7*a***, *lane 2*), Ca ion (**Fig 7*a***, lane 3), and substrate (α_1(XVI) NC11) (**Fig 7*a***, *lane 4*). Plasma fibronectin was cross-linked into high molecular weight complexes (at the top of the gel) by FXIIIa (**Fig 7*b***, *lane 1*).

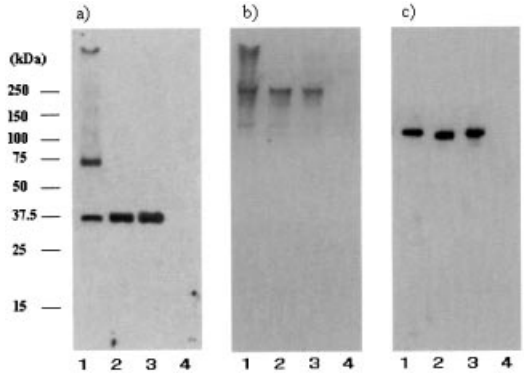

Figure 7. Factor XIIIa-mediated cross-linking of NC11 domain of (*a*) α_1(XVI), (*b*) plasma fibronectin, or (*c*) type III collagen. The products of four different sets of incubation were analyzed by SDS–PAGE followed by western blot. Each incubation was performed for 60 min at 37°C. Incubation 1 (*lane 1*) contains 50 μg protein substrates per ml, 2.5 μg thrombin per ml, 20 μg per ml FXIII and 5 mM $CaCl_2$. Incubation 2 (*lane 2*) lacks thrombin. Incubation 3 (*lane 3*) contains 50 mM EDTA instead of $CaCl_2$. Incubation 4 (*lane 4*) lacked α_1(XVI)NC11. The reaction mixture was heat-denatured in the presence of 1% SDS and 1 mM DTT, and resolved on 5–20% SDS–PAGE. The proteins were transblotted on to the membrane, then incubated with anti-α_1(XVI) NC11 anti-fibronectin or anti-type III collagen antibody.

DISCUSSION

We have demonstrated that $\alpha_1(XVI)^+$ cell populations in the superficial dermis of normal skin contain $FXIIIa^+$ and $FXIIIa^-$ cells. The majority of $\alpha_1(XVI)^+$ cells were found to be $FXIIIa^+$, and a few $\alpha_1(XVI)^+$ cells were $FXIIIa^-$. The population of $\alpha_1(XVI)^-/FXIIIa^+$ was not detected. Similar results were obtained in the lesional skin of dermatofibroma in which tumor cells are considered to be of $FXIII^+$DD origin (Cerio *et al*, 1989b) and psoriatic skin in which increased numbers of $FXIIIa^+$ DD in the papillary dermis have been reported (Cerio *et al*, 1989a). The results suggest that $\alpha_1(XVI)^+$ cell population in the superficial dermal cells mostly corresponds to $FXIIIa^+$ cell population in both normal and diseased conditions and α_1(XVI) collagen can be a molecular marker of $FXIIIa^+$ DD. Clinically, it is often very important to differentiate dermatofibroma from its malignant counterparts, dermatofibrosarcoma and malignant fibrous histiocytoma. In the lesional skin of dermatofibrosarcoma and malignant fibrous histiocytoma, there were no immunoreactive cells with anti-α_1(XVI) collagen antibody (unpublished observation).

The presence of $\alpha_1(XVI)^+FXIIIa^-$cells suggests that connective tissue cells in the upper dermis contain very heterogeneous populations. $\alpha_1(XVI)^+FXIIIa^-$cells in the superficial dermis have a similar phenotypic feature to that of skin fibroblast culture in which fibroblasts express α_1(XVI) collagen (Grassel *et al*, 1996, 1998; Akagi *et al*, 1999; Tajima *et al*, 2000) but do not express FXIIIa as has been shown by reverse transcription–PCR and western blot assays (see **Figs 3*b*** and **4*b***).

We have also demonstrated that expression of α_1(XVI) collagen and FXIIIa at mRNA and protein levels is coordinately enhanced in the dendritic cells differentiated from peripheral blood monocytes *in vitro*. This cell culture system using GM-CSF and IL-4 has been designed to induce pheripheral blood monocytes into $FXIIIa^+$ dendritic cells (Sallusto and Lanzavecchia, 1994), and has provided evidence that DD are originated from monocytes. The induction of α_1(XVI) collagen as well as FXIIIa in this culture system will support this hypothesis. It is of particular interest that a low level of α_1(XVI) collagen mRNA and a few number of $\alpha_1(XVI)^+$ cells were found in the adherent $CD14^+$ peripheral blood monocytes prior to treatment with GM-CSF/IL-4.

The colocalization of FXIIIa and α_1(XVI) in the superficial connective tissue cells *in vivo*, and coordinate expression of FXIIIa and α_1(XVI) in the dendritic cells differentiated from $CD14^+$

monocytes *in vitro* strongly suggest that there is an important biologic relationship between the expression of type XVI collagen and FXIIIa. FXIIIa is considered to catalyze the cross-linking of fibrin and stabilize clot formation at the final stage of coagulation pathway. Although the amino acid sequence of FXIIIa subunit deduced from cDNA sequence does not contain a typical hydrophobic leader sequence for secretion and suggests cytoplasmic protein, the tetrameric plasma enzyme (a2b2) occurs by an unknown secretion mechanism (Muszbek *et al*, 1996; Aeschlimann and Thomazy, 2000). Indeed, we have shown the presence of a considerable amount of FXIIIa in the conditioned medium of the dendritic cells differentiated from monocytes. The physiologic role of FXIII is not restricted to the area of hemostasis, but possibly plays a part in connective tissue organization in wound healing or repair. The previous reports that wound healing is impaired in FXIII-deficient patients (Lorand *et al*, 1980), FXIII stimulates fibroblast proliferation and suppresses collagen biosynthesis by fibroblasts (Beck *et al*, 1961; Paye *et al*, 1989), FXIIIa catalyzes the cross-linking of fibrin(ogen), fibronectin itself, and fibronectin to collagens type I, II, III, and V (Mosher *et al*, 1980; Mosher, 1984) may support this idea. By contrast, it has been demonstrated that FXIIIa, unlike tissue transglutaminase, does not catalyze the cross-linking of aminopropeptide of type III collagen (Bowness *et al*, 1987) or the cross-linking between collagens (Mosher, 1984). Intermolecular cross-linking of α_1(XVI) molecule catalyzed by FXIIIa therefore may be unique in FXIIIa$^+$ DD, suggesting that the cross-linking may stabilize and support the placement of DD in the extracellular tissue. Indeed, Sueki *et al* (1993) have recently showed that DD are surrounded by a unique matrix that may stabilize their placement in the dermis by an electron microscopic observation.

α_1(XVI) molecule consists of 11 noncollagenous and 10 collagenous domains. Except for a large N-terminal NC11 domain of 312 residues, most of the NC domains of α_1(XVI) molecule were very short (11–39 residues). Because nontriple-helical globular domains in FACIT members have been reported to play an essential part in their structural integrity and physiologic function, such as NC1, NC2, and NC3 domains of type IX collagen (Wu *et al*, 1992) and NC1 domain of type XVIII collagen (O'Reilly *et al*, 1997), we produced two recombinant proteins for transglutamination experiments corresponding to N-terminal noncollagenous (NC11) and C-terminal collagenous and noncollagenous (COL1·NC1) domains of α_1(XVI) polypeptide rather than central collagenous domains. We have demonstrated that the NC11 domain of the α_1(XVI) chain is a potential site for FXIIIa-mediated cross-linking. No definite consensus sequence for reactive glutamine residues in the transglutaminase substrates has been determined. The location of reactive glutamine residues in the respective substrate proteins, however, appears to have several common features. When two glutamine residues are directly adjacent or when the glutamine residues are located within 15 amino acids from the NH_2 or COOH terminus, they often potentially function as amine acceptor sites (Aeschlimann *et al*, 1992). Although we did not determine the precise position of reactive glutamine residue in NC11 domain in this study, on the basis of these putative features of the sequences candidate glutamine residues in the NC11 domain are found at amino acid positions 11 and 12 (PSQQEG) from the N-terminal end. The precise studies using the smaller fusion proteins of the NC11 domain are now in progress.

Type XVI collagen has been demonstrated to distribute widely in the skin, heart, kidney, intestine, ovary, testis, eye, arterial wall, and smooth muscle by *in situ* hybridization and immunohistochemical study (Lai and Chu, 1996). FXIIIa$^+$ dendritic cells also ubiquitously occur in collagen-rich tissues, including skin, fascia, tendon, arterial wall, and bone (Headington, 1986; Hoyo *et al*, 1993). Cross-linking of α_1(XVI) collagen mediated by FXIIIa in the DD appears to occur in other dendritic cell-rich tissues as well. But we do not clarify whether FXIIIa is capable of cross-linking the recombinant full-length α_1(XVI) collagen chain. FACIT collagens have been demonstrated to interact with the fibrils of major collagens. For example, the association of type IX collagen with the fibril of type II collagen is demonstrated by the presence of covalent linkages between these two collagens (Wu *et al*, 1992). Type XII and XIV collagens show a remarkable homology with type IX in their C-terminal collagenous domain (COL1). This homology, and consistent codistribution and copurification of type I, type XII, and XIV collagens suggest that these collagens may play a part on type I collagen fibrils similar to the role of type IX on type II collagen fibrils (Keene *et al*, 1991; Nishiyama *et al*, 1994). Indeed, an interaction of collagen XIV with type I collagen fibrils mediated by decorin has been demonstrated (Fout *et al*, 1993). Although the question whether type XVI collagen may interact with other extracellular matrices such as interstitial (type I or III) collagens, fibronectin, or decorin remains unanswered, FXIIIa-mediated stabilization of type XVI collagen may play an essential part in maintaining structural integrity of the FXIIIa$^+$ dendritic cell-rich tissues.

REFERENCES

Aeschlimann D, Paulsson M: Cross-linking of laminin-nidogen complexes by tissue transglutaminase. A novel mechanism for basement membrane stabilization. *J Biol Chem* 266:15308–15317, 1991

Aeschlimann D, Thomazy V: Protein cross-linking in assembly and remodelling of extracellular matrices: The role of transglutaminase. *Connect Tiss Res* 41:1–27, 2000

Aeschlimann D, Paulsson M, Mann K: Identification of Gln^{726} in nidogen as the amine receptor in transglutaminase-catalyzed cross-linking of laminin-nidogen complexes. *J Biol Chem* 267:11316–11321, 1992

Akagi A, Tajima S, Ishibashi A, Yamaguchi N, Nagai Y: Expression of type XVI collagen in human skin fibroblasts: Enhanced expression in fibrotic skin diseases. *J Invest Dermatol* 113:246–250, 1999

Beck E, Duckert F, Ernst M: The influence of fibrin stabilizing factor on the growth of fibroblasts in vitro and wound healing. *Thromb Haemost* 6:485–491, 1961

Bockenstedt P, McDonagh J, Handin RI: Binding and covalent cross-linking of purified von Willebrand factor to native monomeric collagen. *J Clin Invest* 78:551–556, 1986

Bowness JM, Folk JE, Timpl R: Identification of a substrate site for liver transglutaminase on the amino propeptide of type III collagen. *J Biol Chem* 262:1022–1024, 1987

Cerio R, Griffiths CEM, Cooper KD, Nickoloff BJ, Headington JT: Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 121:421–431, 1989a

Cerio R, Spaull J, Jones EW: Histiocytoma cutis: a tumor of dermal dendrocytes (dermal dendrocytoma). *Br J Dermatol* 120:197–206, 1989b

Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987

Fesus L, Metsis ML, Muszbek L, Koteliansky VE: Transglutaminase-sensitive glutamine residues of human plasma fibronectin revealed by studying its proteolytic fragments. *Eur J Biochem* 154:371–374, 1986

Fout B, Aubert-Foucher E, Goldschmidt D, Eichenberger D, van der Rest M: Binding of collagen XIV with the dermatan sulfate side chain of decorin. *J Biol Chem* 268:25015–25018, 1993

Fukai N, Apte SS, Olsen BR: Nonfibrillar collagens. *Methods Enzymol* 245:3–28, 1994

Gibran NS, Nickoloff BJ, Holbrook KA: Ontogeny and characterization of factor XIIIa cells in developing human skin. *Anat Embryol (Berlin)* 193:35–41, 1996

Gilmour TK, Wilkinson B, Breit SN, Kossard S: Analysis of dendritic cell populations using a revised histological staging of morphoea. *Br J Dermatol* 143:1183–1192, 2000

Grassel S, Timpl R, Tan EML, Chu ML: Biosynthesis and processing of type XVI collagen in human fibroblasts and smooth muscle cells. *Eur J Biochem* 242:576–584, 1996

Grassel S, Tan EML, Timpl R, Chu ML: Collagen type XVI expression is modulated by basic fibroblast growth factor and transforming growth factor-β. *FEBS Lett* 436:197–201, 1998

Grassel S, Unsold C, Schacke H, Bruckner-Tuderman L, Bruckner P: Collagen XVI is expressed by human dermal fibroblasts and keratinocytes and is associated with the microfibrillar apparatus in the papillary dermis. *Matrix Biol* 18:309–317, 1999

Grundmann U, Amann E, Zettlmeissl G, Küpper HA: Characterization of cDNA coding for human factor XIIIa. *Proc Natl Acad Sci USA* 83:8024–8028, 1986

Headington JT: The dermal dendrocyte. In: Callen JP, Dahl MV, Golitz LE, Rassmussen JE, Stegmen SJ (eds). *Advances in Dermatology*, Vol. 1. Chicago, IL: YearBook Medical Publishers, 1986, pp 159–171

Henriksson P, Becker S, Lynch G, McDonagh J: Identification of intracellular factor XIII in human monocytes and macrophages. *J Clin Invest* 76:528–534, 1985

Hoyo E, Kanitakis J, Schmitt D: The dermal dendrocyte. *Pathol Biol* 41:613–618, 1993

Kawasaki ES: Amplification of RNA. In: Innis MA, Gelfand DH, Sninsky JJ, White

TJ (eds). *PCR Protocols: a Guide to Method and Applications*. New York: Academic Press, 1990, pp 21–27

Keene DR, Lunstrum GP, Morris NP, Stoddard DW, Burgeson RE: Two type XII-like collagen localized to the surface of banded collagen fibrils. *J Cell Biol* 113:971–978, 1991

Kofeldt E, Maurer P, Vannahme C, Timpl R: Properties of the extracellular calcium binding module of the proteoglycan testican. *FEBS Lett* 414:557–561, 1997

Lai CH, Chu ML: Tissue distribution and developmental expression of type XVI collagen in the mouse. *Tissue Cell* 28:155–164, 1996

Lorand L, Losowsky MS, Miloszewski KJM: Human factor XIII: Fibrin-stabilizing factor. *Prog Hemost Thromb* 5:245–290, 1980

Mosher DF: Cross-linking of fibronectin to collagenous proteins. *Mol Cell Biochem* 58:63–68, 1984

Mosher DF, Schad PE, Vann JM: Cross-linking of collagen and fibronectin by factor XIIIa. *J Biol Chem* 255:1181–1188, 1980

Muszbek L, Yee VC, Hevessy Z: Blood coagulation factor XIII. structure and function. *Thromb Res* 94:271–305, 1996

Nestle FO, Zheng XG, Thompson CB, Turka LA, Nickoloff BJ: Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. *J Immunol* 151:6535–6545, 1993

Nickoloff BJ: The human progenitor cell antigen (CD34) is localized on endothelial cells, dermal dendritic cells, and perifollicular cells in formalin-fixed normal skin and on proliferating endothelial cells and stromal spindle-shaped cells in Kaposi's sarcoma. *Arch Dermatol* 127:523–529, 1991

Nickoloff BJ, Griffiths CEM: The spindle-shaped cells in cutaneous Kaposi's sarcoma. Histologic simulators include factor XIIIa dermal dendrocytes. *Am J Pathol* 135:793–800, 1989

Nishiyama T, McDonough AM, Bruns RR, Burgeson RE: Type XII and XIV collagen fibers mediate interactions between banded collagen fibers in vitro and may modulate extracellular matrix deformability. *J Biol Chem* 269:28193–28199, 1994

O'Reilly MS, Boehm T, Shing T, *et al:* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88:277–285, 1997

Pan TC, Zhang RZ, Mattei MG, Timpl R, Chu ML: Cloning and chromosomal location of human α_1(XVI) collagen. *Proc Natl Acad Sci USA* 89:6565–6569, 1992

Paye M, Nusgens BV, Lapiere CM: Factor XIII of blood coagulation modulates collagen biosynthesis by fibroblasts in vitro. *Haemostasis* 19:274–283, 1989

Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J Exp Med* 179:1109–1118, 1994

Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463–5467, 1977

Sueki H, Whitaker D, Buchsbaum M, Murphy GF: Novel interactions between dermal dendrocytes and mast cells in human skin. Implications for homeostasis and matrix repair. *Lab Invest* 69:160–172, 1993

Tajima S, Akagi A, Tanaka N, Ishibashi A, Kawada A, Yamaguchi N: Expression of type XVI collagen in cultured skin fibroblasts is related to cell growth arrest. *FEBS Lett* 469:1–4, 2000

Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76:4350–4354, 1979

Vuorio E, de Crombrugghe B: The family of collagen genes. *Annu Rev Biochem* 59:837–872, 1990

Weisberg LJ, Shiu DT, Conkling PR, Shuman MA: Identification of normal human peripheral blood monocytes and liver as sites of synthesis of coagulation factor XIIIa-chain. *Blood* 70:579–582, 1987

Wu JJ, Woods PE, Eyre DR: Identification of cross-linking sites in bovine cartilage type IX collagen reveals an antiparallel type II-type IX molecular relationship and type IX bonding. *J Biol Chem* 267:23007–23014, 1992

Yamaguchi N, Kimura S, McBride OW, *et al:* Molecular cloning and partial characterization of a novel collagen chain, α_1(XVI), consisting of repetitive collagenous domains and cysteine-containing non-collagenous segments. *J Biochem (Tokyo)* 112:856–863, 1992

Yamaguchi N, Anand-Apte B, Lee M, *et al:* Endostatin inhibits VEGF-induced endothelial cell migration and tumor growth independently of zinc binding. *EMBO J* 18:4414–4423, 1999

Yoo YH, Park BS, Whitaker-Menezes D, Korngold R, Murphy GF: Dermal dendrocytes participate in the cellular pathology of experimental acute graft-versus host disease. *J Cutan Pathol* 25:426–434, 1998

Young DA, Lowe LD, Clark SC: Comparison of the effects of IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor in supporting monocyte differentiation in culture. *J Immunol* 145:607–615, 1990