Expression of Type XIV Collagen During the Differentiation of Fetal Bovine Skin: Immunolabeling with Monoclonal Antibody Is Prominent in Morphogenetic Areas

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Type XIV collagen belongs to the subclass of fibril-associated collagens with interrupted triple helices, which are composed of alternative triple helical and non-collagenous domains. Structural data show that these molecules interact with collagen fibrils and suggest that they might interact with cells. We have investigated the expression of type XIV collagen in bovine skin during development. Fetuses from 9 to 37 weeks were examined. Anti-type XIV collagen monoclonal antibody was produced, characterized, and used for immunofluorescence detection of the molecule. The localization of immunolabeling was analyzed by comparison with light and electron microscopic observations. In 9-week-old fetus, no type XIV collagen was found in the skin. From 19 weeks to birth, extensive immunofluorescence was observed on bundles of collagen fibrils in deep dermis. As shown by electron microscopy, this area exhibited bundles of collagen fibrils and cells with an abundant rough endoplasmic reticulum. In the upper dermis, a delicate fibrillar network of type XIV collagen was revealed by immunofluorescence around growing hair follicles at 19 and 24 weeks. Double labeling for type XIV collagen and fibronectin shows a more restricted pattern of expression of type XIV collagen in this area. The electron microscopic examination of skin of fetuses at these stages shows that the whole upper dermis is composed by a loose connective tissue containing scattered small bundles of collagen fibrils. Type XIV collagen was synthesized in the upper dermis between 24 weeks and birth. From this study, it appears that type XIV collagen expression is distinct from that of fibrillar collagens, at least during some developmental events. The prominent localization of type XIV collagen around growing hair follicles suggests a role for this molecule in epithelial-mesenchymal interactions.
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The collagen family of proteins comprises some 15 members [1]; the functions for several of them are not yet clearly understood. The newly described type XIV collagen [2,3] belongs to a subclass with unique structural characteristics, named the FACIT group (fibril-associated collagens with interrupted triple helices) by Olsen [4]. Three FACIT collagens are known at present, types IX, XII, and XIV, the best characterized being type IX collagen, associated with type II collagen fibrils [5,6]. Immunostaining with a monoclonal antibody specific for type XII collagen demonstrated that these molecules are associated with type I collagen fibrils in certain dense connective tissues such as tendons, ligaments, and perichondrium [7]. A distinct but closely related molecule, type XIV collagen, was isolated from type I collagen–containing tissues such as skin and tendons [2,3]. Considering the association of FACIT collagens with collagen fibrils and assuming that the organization of type IX collagen at the surface of fibrils [8] as a general scheme, it has frequently been postulated that the FACIT collagens constitute molecular bridges, linking together the fibrils of a tissue [9,10]. The generalization of this organization has been strongly supported by the immunolocalization of two collagen molecules related to type XII [11] at the surface of collagen fibrils [12]. However, an additional function of at least some of the FACIT collagens in cell-matrix interactions has been suggested by the knowledge of the complete primary structure of chicken type XII collagen; a cDNA analysis of this collagen has revealed a complex succession of protein motifs (fibronectin type III, von Willebrand factor A) in the non-helical amino-terminal end, and an arginin-glycin-aspartic acid (RGD) potential cell recognition adhesive sequence in a helical domain [13]. A similar modular structure was recently described for type XIV collagen [14]. These structural data indicate that these molecules may interact with other matrix components and with matrix receptors present on cell surfaces. One approach for revealing the functions of such molecules is to study their developmental expression. With this prospect, using a monoclonal antibody specific to bovine type XIV collagen obtained in the laboratory, we have investigated the localization of this FACIT collagen in various stages of fetal bovine skin.

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Abbreviations: ABTS, 2,2′-azino-di(3-ethyl-benzthiazoline sulfonate); CAPS, (3-[cyclohexylamino]-1-propanesulfonic acid); FACIT, fibril-associated collagen with interrupted triple helices; RGD, arginin-glycin-aspartic acid.

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MATERIALS AND METHODS

Reagents Unless otherwise specified, chemicals were purchased from Sigma (St Louis, MO) and tissue culture reagents from Gibco Laboratories (UK).

Antigen Preparation Bovine type XII and type XIV collagens were purified respectively from tendon and skin of 6-month-old fetuses following a previously described procedure [15]. Briefly, tissues were first extracted with 0.25 M NaCl. The resulting extract was submitted to carboxymethyl (CM)-cellulose chromatography and further purified by chromatography on Con A-sepharose. Differential elution of type XIV and type XII collagens was achieved respectively with α-methyl mannoside and α-methylglucopyranoside.

Immunization A five-week-old female Balb/c mouse was immunized with bovine type XIV collagen purified from skin. Ten micrograms of antigen emulsified in complete Freund's adjuvant were injected subcutaneously at multiple sites. Booster injections were given 3 and 5 weeks later with the same dose in incomplete adjuvant. Intraperitoneal boosts with antigen in Con A elution buffer were done 8 and 1 d before fusion.

Monoclonal Antibody Production The methods for fusion and establishment of antibody-producing hybridomas followed the procedures described by Linsenmayer and Hendrix [16]. The mouse was sacrificed by cervical dislocation and the spleen was removed aseptically. The resulting cell suspension was fused with sp2/0 Ag 14 myeloma with polyethyleneglycol 4000 at a ratio of three splenocytes to one myeloma cell. After fusion, the cells were dispersed into 96-well plates. Antibody secretion was screened by enzyme-linked immunosorbent assay (ELISA) (see above) against purified type XIV collagen (0.1 μg/well). Positive hybridomas were cloned twice by the method of limiting dilution. Hybridoma culture medium was composed of RPMI supplemented with 1 mM pyruvate, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml glucose, 0.05 mM mercaptoethanol, 10 mM (HEPES), (N-[2-hydroxyethyl]piperazine-N'-2-ethanesulfonic acid (HEPES), and 10% fetal calf serum.

Elisa For screening anti-type XIV collagen producing hybridomas, and for subsequent testing of antibody specificity, an ELISA technique was used. Polystyrene microtiter plates (Greiner, France) were coated with 100 μl of antigen diluted in phosphate-buffered saline (PBS) and dried under vacuum (RC 10-01, Jouan, France). Non-specific sites were blocked with 1% bovine serum albumin (BSA), 0.05% Tween 20 in PBS. The antibody to be tested was diluted if necessary in the blocking solution, added to the wells for 1 h, and rinsed with 0.05% Tween in PBS. Wells were incubated in peroxidase-conjugated anti-mouse Ig (Dakopatts, Denmark) and rinsed. The amount of bound enzyme was measured with 2,2'-azino-di(3-ethyl-benzthiazoline sulphonate) (ABTS) and the absorbance read at 405 nm (SLT Instruments microplate photometer).

Characterization of Monoclonal Antibodies by Immunoblotting A crude preparation containing type XIV collagen was obtained by extracting bovine skin with 0.25 M NaCl in the presence of protease inhibitors, according to Aubert-Foucher *et al* [15]. The final protein concentration of this extract was approximately 1 mg/ml. For disulfide bond reduction, samples were treated with 2 mM dithiothreitol. After denaturation (3 min at 100°C), optional alkylation with 0.2 M iodoacetamide was performed 1 h in the dark. The extracts were then dialyzed for 24 h at 4°C against collagenase buffer (20 mM Tris, pH 7.6; 0.2 M NaCl; 5 mM $CaCl_2$). Some samples were treated with collagenase (Advanced Biofacture) at 250 U/ml. An equivalent volume of buffer was added in controls. Digestion was carried out for 24 h at 37°C.

SDS-PAGE was performed in Mini-Protean II system (BioRad) according to Laemmli [17]. Sample buffer (5× concentrated) was added to the 0.25 M NaCl extracts and boiled for 3 min. Five microliters of extract were loaded on 6% acrylamide gels and transferred electrophoretically to immobilon membrane (Millipore) in 10 mM (3-[cyclohexylamino]-1-propanesulfonic acid CAPS), pH 11, 5% methanol for 5 h at 60 V.

The membrane was treated with 10% low-fat dried milk in PBS, incubated with antibodies (hybridoma supernatant diluted 1:100 in Tween-PBS) for 1 h, and rinsed. The primary antibody was detected with anti-mouse Ig conjugated with alkaline phosphatase (Dakopatts, Denmark) diluted 1/1500 and rinsed in PBS. The membrane was treated with an alkaline-phosphatase detection kit (BioRad), rinsed in water, and dried.

Tissue Specimens Fetal bovine skins were obtained from the local slaughterhouse within 2 h after death. Age of fetuses was estimated with the femoral or tibial length, according to Pal *et al* [18].

Immunofluorescence Samples were embedded in OCT compound (Miles Scientific, Naperville, IL), frozen in liquid nitrogen, and stored at −70°C. Sections (6 μm thick) made with a cryostat were harvested on aminoalkylsilane-treated slides [19] and air-dried.

After incubation in 1% bovine serum albumin (BSA) in PBS, the primary antibody (hybridoma supernatant) was added in concentrations ranging from undiluted to 320-times dilution. Immunoglobulins fixed to the antigen were recognized with anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC)-diluted 1:400 (Biosys, France). For double-labeling experiments, the second antigen was detected by polyclonal rabbit anti-human type IV collagen, anti-bovine type I collagen, or anti-human fibronectin (IgG fractions), diluted 1:100 (Institut Pasteur, Lyon, France) and revealed by rhodamine-linked anti-rabbit IgG diluted 1:100 (Nordic Immunology, The Nederlands). Antibodies and conjugates were diluted in 1% BSA in PBS. Slides were mounted in glycerol/PBS and examined with a Zeiss Universal microscope equipped with epifluorescence. As control, specific antibodies were replaced by purified non-immune mouse IgG (Biosys, France) diluted at 50 μg/ml in hybridoma culture medium or by normal rabbit serum.

To locate more precisely the immunolabeling, some cryosections were stained with 1% toluidine blue, dried, mounted, and observed with a Leitz light microscope.

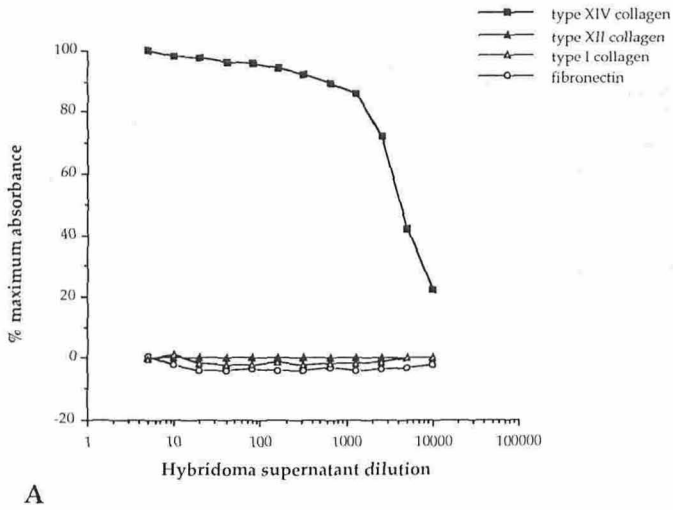
Light and Transmission Electron Microscopy After Epon Embedding Skin samples were fixed in 2% glutaraldehyde in PBS for 3 h at room temperature and rinsed in PBS overnight. Post-fixation was performed during 1 h in 1% osmium tetroxide in PBS. Tissues were then dehydrated in graded ethanol solutions and embedded in Epon 812. Sections were obtained using a Reichert-Jung Ultracut microtome.

Semi-thin sections were mounted on glass slides, stained with methylene blue-azur II, and photographed with a Leitz light microscope.

Thin sections were contrasted with methanolic uranyl acetate and lead citrate. Grids were examined with a Jeol 1200EX electron microscope at the Centre de Microscopie Electronique Appliquée à la Biologie et à la Géologie (Claude Bernard University, Villeurbanne, France).

RESULTS

Specificity of Monoclonal Antibody to Type XIV Collagen The monoclonal antibody used in this study displayed significant reactivity in ELISA with purified type XIV collagen, with a titer of 5×10^3 for hybridoma culture supernatant (Fig 1*A*). Under the same conditions, type XII collagen, a closely related molecule, was not detected. Other extracellular matrix molecules such as fibronectin and type I collagen were not recognized (Fig 1*A*). Using a crude preparation, 0.25 M NaCl skin extract, we showed that on immunoblots after sodium dodecylsulfate-polyacrylamide gel electrophoresis the pattern of the antibody's reactivity is consistent with the electrophoretic characteristics of type XIV collagen (Fig 1*B*). Under non-reducing conditions, high-molecular-mass components are recognized: the bands are located at the upper limit of the 6% polyacrylamide separating gel. After reduction, the two charac-



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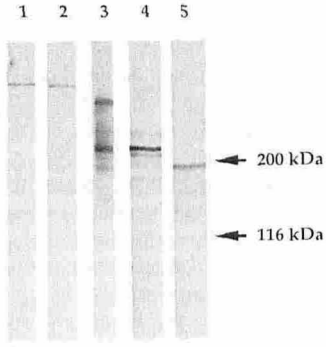

B

Figure 1. Characterization of the monoclonal antibody CY15B8. *(A)* Titration curves of hybridoma culture supernatant. Plates were coated with 5 ng of antigen per well and the reactivity measured by ELISA as described in *Materials and Methods.* Antigens used were bovine type XIV collagen (■), type XII collagen (▲), type I collagen (△), and fibronectin (○). *(B)* Immunoblot against 0.25 M NaCl skin extract separated on 6% polyacrylamide gel. The CY15B8 hybridoma culture supernatant was diluted 1:100. *Lane 1,* unreduced sample; *lane 2,* same as in *lane 1,* after treatment with collagenase; *lane 3,* reduced sample; *lane 4,* reduced and alkylated sample; *lane 5,* same as in *lane 4,* treated with collagenase.

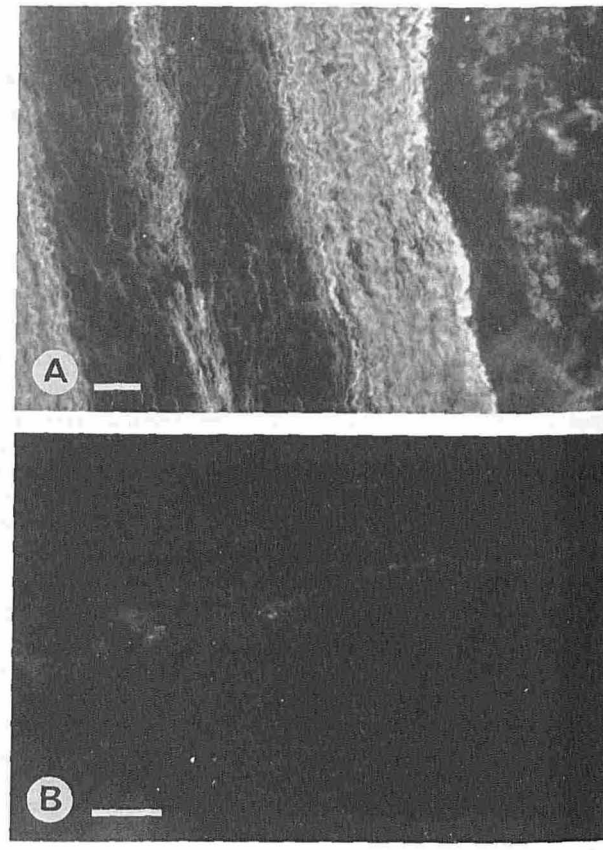

Figure 2. Type XIV collagen localization in 9-week-old bovine fetus. In tendon *(A)* bundles of collagen fibers display a good reactivity, whereas in skin *(B)* labeling is undetectable. *Bar,* 50 μm.

teristic bands of type XIV collagen, at 220 and 290 kDa, are labeled. Additional alkylation with iodoacetamide causes the appearance of one unique band at 220 kDa. This observation is consistent with the previous demonstration of one cryptic disulfide bond between two alpha chains of type XIV collagen [15]. After digestion with collagenase, the antibody reacts with a band at 190 kDa, indicating that its epitope is located in the NC3 domain of type XIV collagen.

Sites of Type XIV Collagen Expression In a 9-week-old bovine fetus, type XIV collagen was clearly detected in large bundles of collagen fibrils in tendons (Fig 2*A*), whereas it could not be detected in the skin (Fig 2*B*) at this stage.

In a 19-week-old fetus, type XIV collagen was evident in the skin. In the deep dermis, a faint staining was observed on large bundles of collagen fibrils (Fig 3*A,B,D*) and intracellular accumulations were regularly noticed (Fig 3*B*). In the upper dermis, the detection of type XIV collagen was restricted to the junctional area between dermis and epidermis. It was noticeable around the growing hair follicles (Fig 3*A,F*), where it formed basket-like structures (Fig 3*C*). Occasionally, type XIV collagen was detected in perineurium and endoneurium of nerve endings (Fig 3*D*). Double-labeling with anti-type IV and anti-type XIV collagen antibodies showed their distinct organization in the upper dermis. Type IV collagen was detected at the level of basement membranes, beneath the epidermis, and around the hair follicles (Fig 3*E*). Type XIV collagen was not distinctly associated with the dermal-epidermal basement membrane, but was found underneath it and associated with the collagen bundles surrounding hair follicles (Fig 3*F*). This general distribution of type XIV collagen was retained and enhanced in the skin of fetuses of 22 weeks (Fig 4*A*), 24 weeks (Fig 5*A,B*), and 27 weeks (not shown).

To correlate the expression of type XIV collagen with the structure of bovine developing skin, we performed immunolabeling, and light and electron microscopy on the same skin sample, obtained from a 22-week-old fetus (Fig 4). Low-magnification immunofluorescence (Fig 4*A*) showed that the deep dermis is extensively labeled, compared to the underlying loose connective tissue. This localization of labeling in deep dermis was confirmed by the observation of stained cryosections (Fig 4*B*), where condensation of mesenchymal cells was observed in this area. By observation of semithin sections (Fig 4*C*), we have investigated the structure of developing skin at a higher resolution. Budding hair follicles were surrounded by condensations of dermal cells and the upper dermis was composed of relatively loose connective tissue, compared to deep dermis in which bundles of collagen fibrils and fibroblastic cells were clearly identified. By electron microscopy, deep dermis appeared composed of large bundles of collagen fibrils (50 to 100 nm in diameter) and cells containing extended rough endoplasmic reticulum profiles (Fig 4*D*). In the upper dermis, we found sparse small bundles of collagen fibrils (smaller than 50 nm in diameter) and restrained rough endoplasmic reticulum in cells (Fig 4*E*). Around elongated hair follicles, in the area corresponding to the

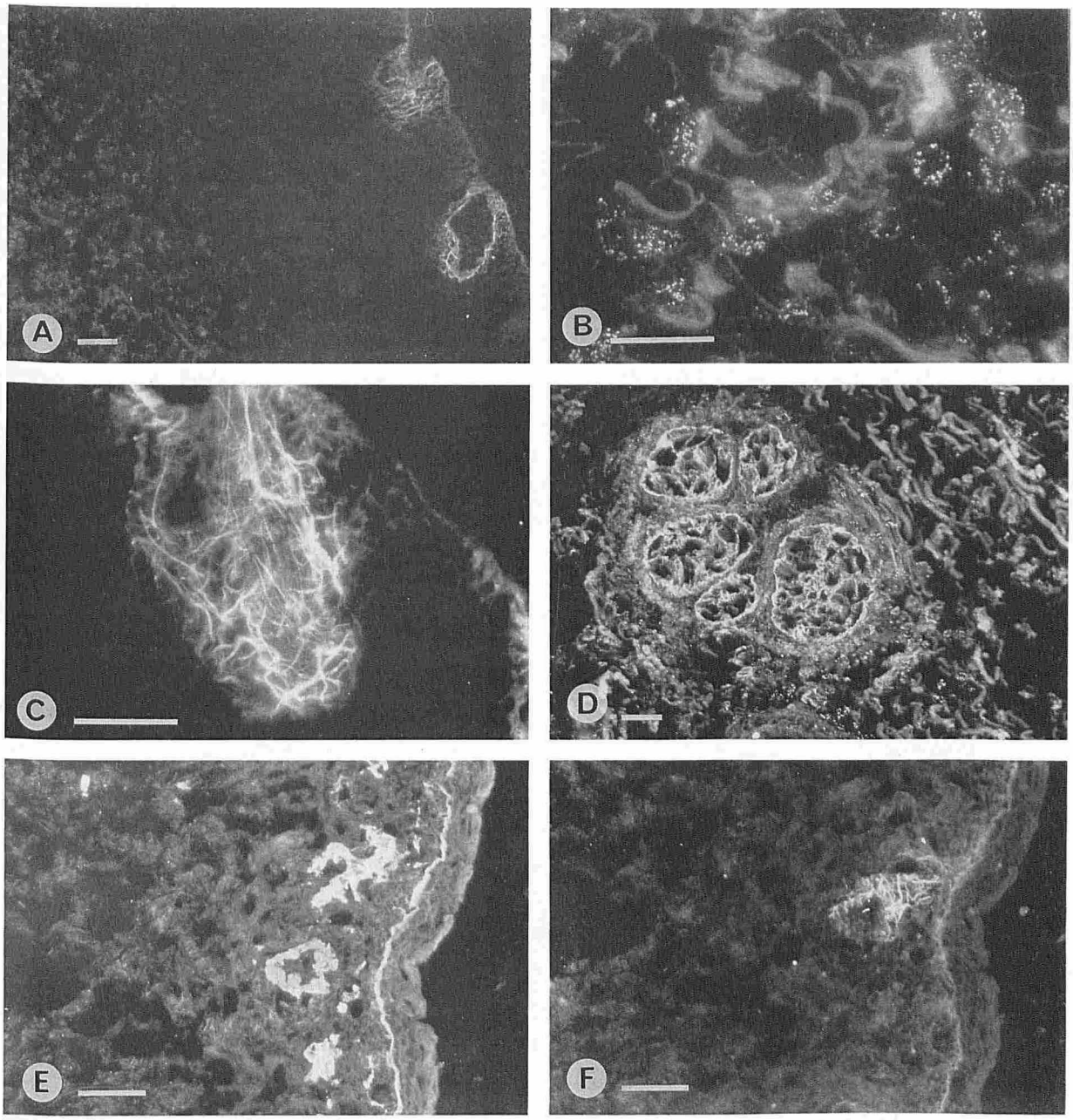

Figure 3. Type XIV collagen localization in 19-week-old bovine fetal skin. Deep dermis and budding hair follicles are intensely labeled *(A)*. In deep dermis, type XIV is present on bundles of collagen fibers and in intracellular vesicles *(B)*, and in perineurium and endoneurium *(D)*. In hair follicles, fluorescence appears as a fibrillar network *(C)*. Double immunolabeling of 19-week-old fetal skin (*E* and *F*). Anti-type IV collagen antibodies were revealed by rhodamine *(E)* and anti-type XIV collagen antibody by FITC *(F)*. The two molecules exhibit distinct localizations in the upper dermis. *Bar,* 50 μm.

localization of type XIV collagen, small bundles of collagen fibrils were observed, beneath keratinocyte basement membrane (Fig 4*F*).

Double-labeling experiments, performed on sections of skin from a 24-week-old fetus showed a distribution of type XIV collagen (Fig 5*A,B*) distinct from that of type I collagen (Fig 5*C*), and from the distribution of fibronectin (Fig 5*D*). Type I collagen was uniformly distributed in the whole dermis (Fig 5*C*), whereas fibronectin was concentrated in the upper dermis including the surroundings of hair follicles and blood vessels (Fig 5*C*). Electron microscope observations performed on this skin sample showed a structure similar to that observed at 22 weeks (Fig 5*E,F*). However, in deep dermis (Fig 5*E*) and around hair follicles (Fig 5*F*), the bundles of collagen fibrils were larger in size.

The concentration of type XIV collagen around the hair follicles appeared to vary according to the developmental stage considered. At 19 weeks, the hair follicles were formed and the type XIV localization was almost uniform (Fig 3*C*). At later stages, type XIV collagen appeared more concentrated in the sheath around the hair follicles (Fig 5*A*). At 37 weeks, just before birth, type XIV collagen immunolabeling was intense and its distribution was uniform in the whole dermis (Fig 6*A,B*).

DISCUSSION

The normal embryonic development of a large number of organs is controlled by the reciprocal interactions of their epithelial and mesenchymal components [20-22]. This multistep inductive process has been particularly documented during the early development of skin appendages (hairs, feathers, and scales), teeth, and branched organs (lung, salivary, and mammary glands). The basement membrane, located at the epithelial-mesenchymal interface, has been

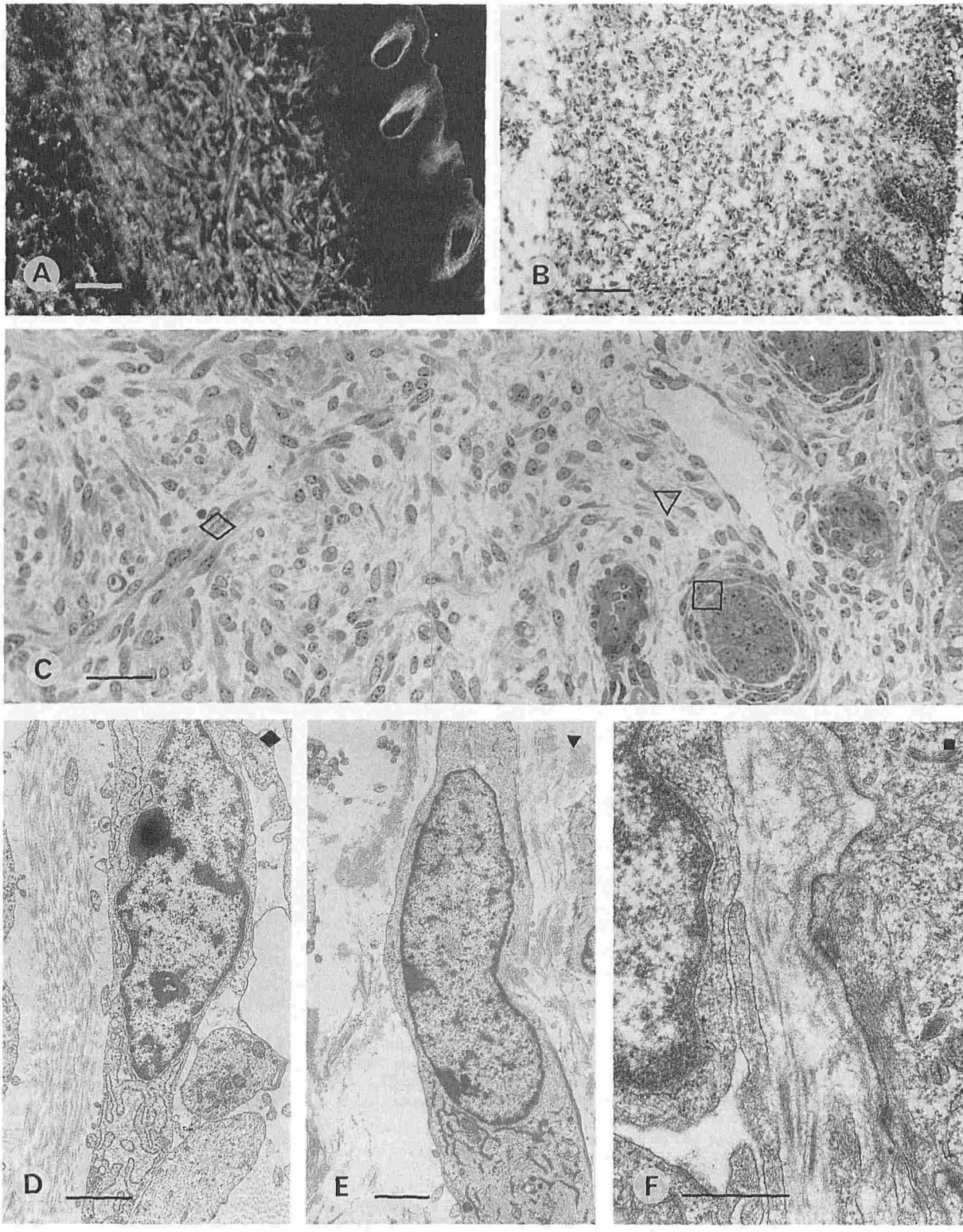

Figure 4. Structure and labeling of 22-week-old fetal skin. Immunofluorescence of type XIV collagen *(A)* is positive around budding hair follicles and in deep dermis. Hypodermal loose connective tissue is faintly labeled. On stained cryosections *(B)*, accumulation of mesenchymal cells is observed in the whole dermis. On semi-thin sections obtained after Epon embedding *(C)*, the budding hair follicles are surrounded by dermal cells accumulation and bundles of collagen fibrils and fibroblastic cells are found in deep dermis. Spotted areas in C correspond to the respective positions in *D*, *E*, and *F*. As seen in transmission electron microscopy (*D*, *E*, and *F*), deep dermis cells exhibit a distended rough endoplasmic reticulum; the visible collagen fibrils are 50 to 100 nm in diameter *(D)*. In the loose connective tissue of upper dermis small bundles of thin fibrils (<50 nm) are observed *(E)*. Similar fibrils are found below basement membrane, around growing hair follicles *(F)*. *Bars: A* and *B*, 100 μm; *C*, 50 μm; *D* and *E*, 2 μm; *F*, 0.5 μm.

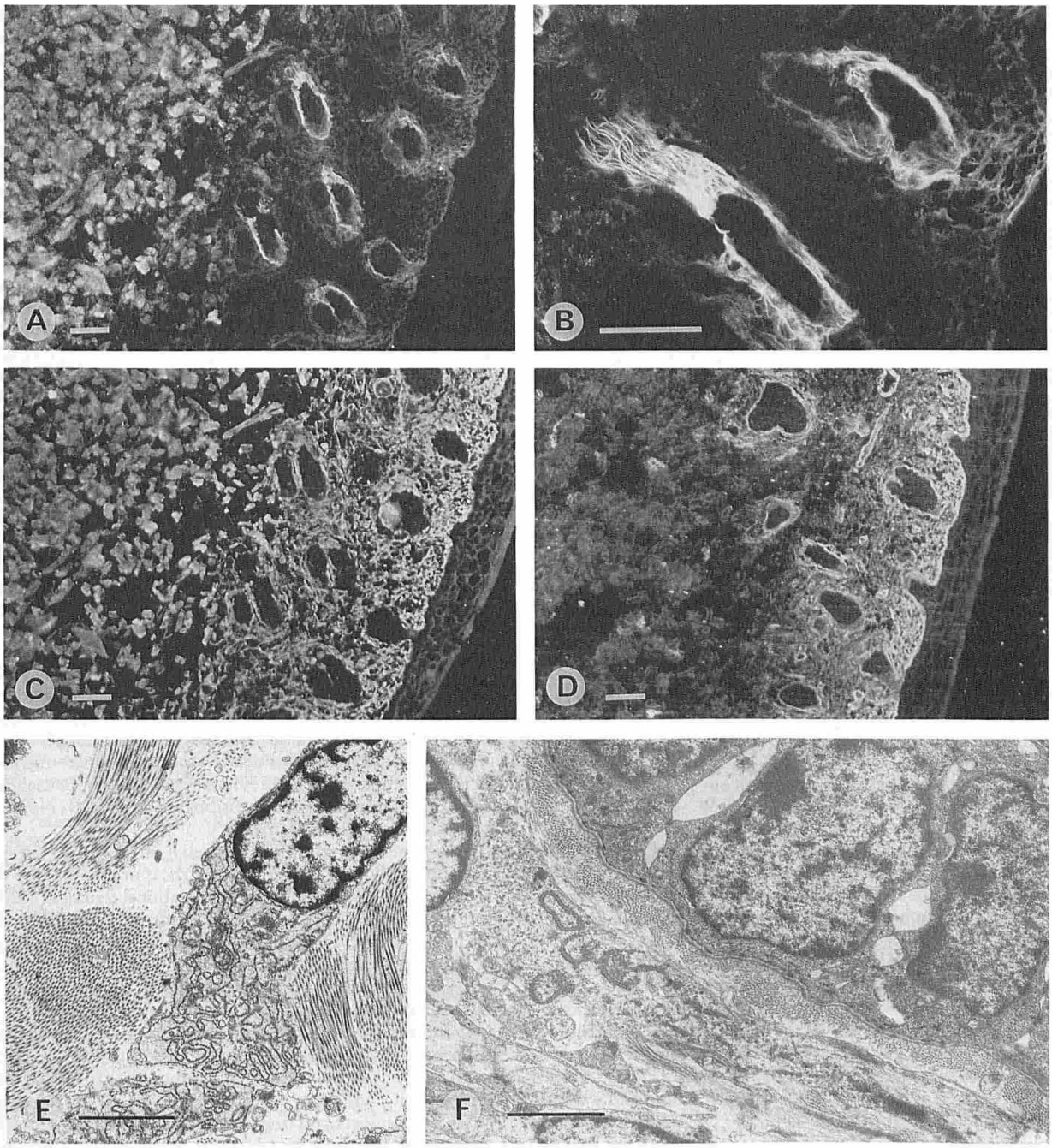

Figure 5. Double immunolabeling and electron microscopy of 24-week-old fetal skin. Deep dermis and the sheath of hair follicles are labeled *(A)*. Enlargement of these follicles shows a filamentous organization of type XIV collagen *(B)*. *(A, C)* Double immunolabeling for type XIV collagen *(A)* and type I collagen *(C)*. Type I collagen is equally distributed in this tissue. Immunolabeling for fibronectin *(D)* shows that its expression is restricted to the upper dermis, with a prominent localization around hair follicles and blood vessels. By electron microscopy, deep dermis is composed of large bundles of collagen fibrils *(E)* whereas closely packed thin collagen fibrils are observed around budding hair follicles *(F)*. *Bars: A, B, C,* and *D*, 50 μm; *E* and *F*, 2 μm.

considered in the mediation of tissue interactions as stabilizing the epithelial differentiation [23]. Several extracellular matrix components are involved during such interactions. Laminin promotes cell growth during lung morphogenesis [24]. Tenascin, a large, six-armed chimeric molecule modulating cell-matrix adhesion [25], is transiently expressed during the development of feather buds [26], hair follicles [27], and scales [28]. Syndecan, a cell surface proteoglycan, is expressed during tooth embryogenesis [29,30]. During branching morphogenesis, the remodeling of the basement membrane is controlled by mesenchyme, which degrades glycosaminoglycans [31]. Collagen fibrils are involved in the stabilization of basement membrane in the interlobular clefts of these branched organs [32,33]. During the development of cutaneous appendages, collagen is usually found in stable areas, whereas fibronectin is located in morphogenetic zones [34,35]. Our results concern a collagen molecule that is thought to be associated with collagen fibrils and consequently could be the actual molecule involved when cells interact *in vivo* with bundles of collagen fibrils.

The previously described procedure of collagen preparation clearly separates type XII and type XIV collagens [15]. The antigen used for monoclonal antibody production was strictly identified as type XIV collagen by protein sequencing. Our antibody, CY 15 B8, recognizes type XIV collagen bands separated by electrophoresis, and, after collagenase digestion, binds to a sub-band corresponding

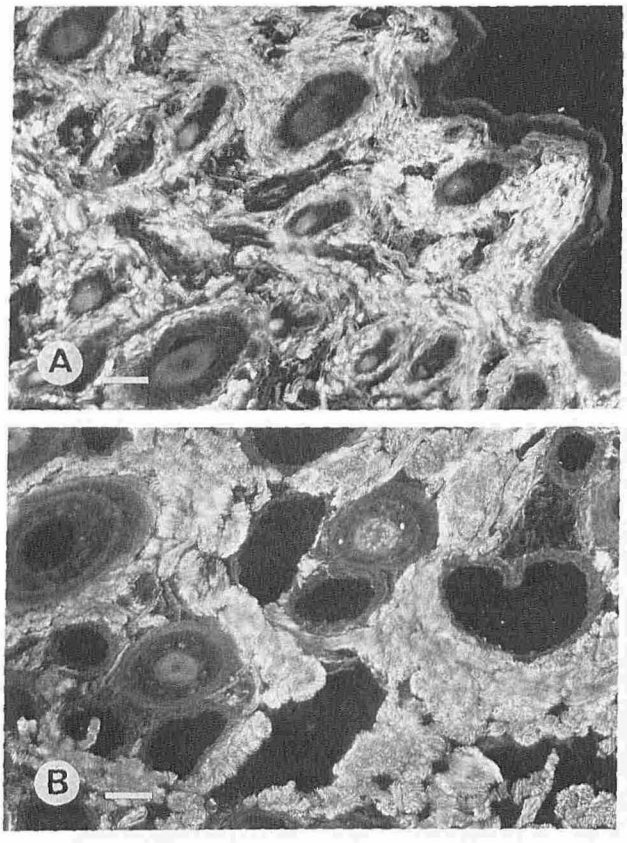

Figure 6. Type XIV collagen localization in 37-week-old fetal skin. The molecule is present in the whole matrix of upper dermis *(A)* and in area of the deeper dermis *(B)*. *Bars,* 50 μm.

to the amino-terminal NC3 domain. It does not cross-react with type XII collagen, type I collagen, and fibronectin. With this narrow specificity and its high ELISA titer, this antibody appears to be a valuable tool for immunofluorescence study of type XIV collagen expression.

The expression of type XIV collagen in bovine embryonic skin is a late and sequential event. At 9 weeks of development, type XIV collagen was not detected in the whole skin, whereas it was clearly visible in tendons. It appeared first in the deep, reticular dermis and around forming hair follicles and was synthesized throughout the dermis just before birth. Its distribution is distinctly different from that of type I collagen and fibronectin. Comparing our results with the data obtained by Lunstrum *et al* [11], the localization of type XIV collagen is similar to the localization of the type XII-like B molecule. Very recently, these authors have confirmed by peptide-sequencing studies that type XII-like B collagen is in fact type XIV collagen [36]. Additionally, the accumulation of intracellular type XIV collagen strongly suggests a massive gene expression in dermal cells, at least at certain stages of development. This observation is consistent with electron microscope data. Indeed in deep dermis during development, cells exhibit a prominent distended rough endoplasmic reticulum, indicating a high level of protein synthesis. As shown by comparison of immunofluorescent and stained cryosections, this deposition of type XIV collagen in skin is concomitant with the differenciation of deep dermis in relation to the underlying loose connective tissue. Also at this stage, we observed a marked difference in the labeling of deep and upper dermis. Assuming that bovine and human gestational periods are similar (274 and 252 d, respectively), these differences could be correlated with the distinction of reticular and papillary dermis that became possible at 4 months in human embryo [37]. It should be emphasized that this elaboration of type XIV collagen is associated with large bundles of type I collagen fibrils, suggesting that it could be involved in the stabilization of extracellular matrix in this area.

During differentiation of embryonic dermis, collagen fibrils appear early in the extracellular matrix, even before the budding of hair follicles. These fibrils are visible by 5 weeks in the human embryo [37]. We confirmed this observation on 18-week-old bovine skin, where hair follicles are not formed; the dermis is mainly cellular, but contains small bundles of collagen fibrils (data not shown). At 22 and 24 weeks in our model, we found numerous collagen fibrils in the upper dermis. These fibrils exhibit the same diameter, all around hair follicles, in interfollicular dermis, whereas type XIV collagen is preferentially deposited around hair follicles. Thus, there is apparently no relationship between the size of collagen fibrils and the presence of type XIV collagen.

The specific localization of type XIV collagen around developing hair follicle strongly suggests a function of this protein during the formation of skin appendages. Interactions between epidermis and dermis are essential for hair follicle initiation and development [38]. The prominent location of type XIV collagen in developing hair follicle mesenchyme is particularly significant in this respect. The molecular composition of extracellular matrix is thought to play an important role in epidermal-mesenchymal interactions. Mauger *et al* [35] reported during hair development of mouse skin an heterogeneous distribution of type I, type III collagens, and fibronectin: collagens became sparse around hair buds and fibronectin was particularly abundant along the dermal-epidermal junction of hair rudiments as well as underneath hair buds. In the bovine model, type I collagen was found uniformly throughout dermis during the formation of hair follicle. Similar results were obtained for type III collagen (data not shown). In our system, the presence of fibrillar collagen seems not related to stabilized zones. Small bundles of collagen fibrils in this area could be sufficiently loose to allow morphogenetic movements. Fibronectin expression during bovine skin development is similar to that observed in mouse; this molecule is abundant in morphogenetically active zones and most likely contributes to cell migration. Fibronectin accumulation around blood vessels is consistent with the previous results of Tonnensen *et al* [39], who observed fibronectin expression during the development of the microvasculature in human skin.

During the invagination of epithelial cells forming the hair follicle, the underlying extracellular matrix is probably a site of extensive turnover. Tissue inhibitor of metalloproteinases (TIMP) gene expression in the sheath of follicle that form vibrissae [40] is significant in this respect. Reponen *et al* [41] have shown that epithelial cells from the hair follicle express the 72-kDa type IV collagenase. In this situation, it could be postulated that keratinocytes may locally interact with dermal connective tissue, considering that the basement membrane is constantly remodeled. The restricted distribution of tenascin was described in this area [27,42]. It has been shown that *in vitro*, this molecule can modulate the behavior of cells by preventing adhesion to fibronectin [43]. The migration of a human keratinocyte cell line on interstitial collagen type I has been established and characterized *in vitro* [44]. Considering the association of type XIV collagen with collagen fibrils, it is tempting to hypothesize that type XIV collagen might prevent also the interaction of cells with fibrillar collagen. Finally, during the morphogenesis of hair follicles, type XIV collagen might have a dual function: 1) the limitation of keratinocyte migration by the partial stabilization of local extracellular matrix and 2) the lowering of keratinocyte attachment to collagen to promote morphogenetic movements. *In vitro* studies using cells isolated from skin would be helpful in the complete understanding of this phenomenon.

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