Induction of Tenascin in Healing Wounds

Eleanor J. Mackie, Willi Halfter, and Dagmar Liverani

Friedrich Miescher-Institut, Basel, Switzerland

Abstract. The distribution of the extracellular matrix glycoprotein, tenascin, in normal skin and healing skin wounds in rats, has been investigated by immunohistochemistry. In normal skin, tenascin was sparsely distributed, predominantly in association with basement membranes. In wounds, there was a marked increase in the expression of tenascin at the wound edge in all levels of the skin. There was also particularly strong tenascin staining at the dermal-epidermal junction beneath migrating, proliferating epidermis. Tenascin was present throughout the matrix of the granulation tissue, which filled full-thickness wounds, but was not detectable in the scar after wound contraction was complete. The distribution of tenascin was spatially and temporally different from that of fibronectin, and tenascin appeared before laminin beneath migrating epidermis. Tenascin was not entirely codistributed with myofibroblasts, the contractile wound fibroblasts. In EM studies of wounds, tenascin was localized in the basal lamina at the dermal-epidermal junction, as well as in the extracellular matrix of the adjacent dermal stroma, where it was either distributed homogeneously or bound to the surface of collagen fibers. In cultured skin explants, in which epidermis migrated over the cut edge of the dermis, tenascin, but not fibronectin, appeared in the dermis underlying the migrating epithelium. This demonstrates that migrating, proliferating epidermis induces the production of tenascin. The results presented here suggest that tenascin is important in wound healing and is subject to quite different regulatory mechanisms than is fibronectin.

WOUND healing is a complex series of biological events that in many ways resembles the process of development. Cellular proliferation, migration, and differentiation, as well as tissue remodeling, are all events that occur during embryonic and postnatal development and recur during wound healing. Many of the molecular mechanisms responsible for these events are apparently common to development and wound healing. Some growth factors that are known or thought to promote wound healing also have roles in development, for example, the fibroblast growth factors (reviewed by Thomas, 1987) and transforming growth factor-β (Mustoe et al., 1987; Seyedin et al., 1986). Some extracellular matrix molecules (e.g., fibronectin and collagen type III) that are abundant in embryonic skin and less abundant in adult skin, are reexpressed at high levels in healing skin wounds (Kurkinen et al., 1980; review by Alvarez, 1986).

Extracellular matrix proteins are known to be important in many phases of wound healing. Fibronectin is present in the clot that forms at the earliest stage of healing and is abundant in the granulation tissue that fills a deep skin wound (Grinnell et al., 1981). Fibronectin is thought to aid in phagocytosis of wound debris and promotes migration of wound fibroblasts, endothelial cells, and probably epithelial cells over the wound (reviewed by Murphy-Ullrich and Mosher, 1986). Collagen type III is increased in the early granulation tissue and is later replaced by collagen type I. These two collagen types impart strength to the healing wound (reviewed by Cohen and McCoy, 1983). Collagen and fibronectin are also chemotactic for fibroblasts (reviewed by Alvarez, 1986).

Tenascin, also known as myotendinous antigen, glioma mesenchymal extracellular matrix antigen, hexabrachion, brachionectin, J1, and cytotactin, is an extracellular matrix glycoprotein with a strikingly limited distribution in the mesenchymal components of various organs in vertebrate embryos (Chiquet and Fambrough, 1984*a*; Chiquet-Ehrismann et al., 1986; Bourdon et al., 1983; Vaughan et al., 1987; Erickson et al., 1987; Faissner et al., 1988; Hoffman et al., 1988). Its tissue distribution suggests that tenascin is important in the development of mammary gland, hair follicle (Chiquet-Ehrismann et al., 1986, Inaguma et al., 1988), muscle (Chiquet and Fambrough, 1984*a*), tooth (Thesleff et al., 1987), cartilage and bone (Mackie et al., 1987*b*), kidney (Aufderheide et al., 1987), and neural crest and its derivatives (Mackie et al., 1988; Epperlein et al., 1988), as well as the central nervous system (Kruse et al., 1985). Tenascin is absent or expressed at low levels in the adult mammary gland, but is abundant in mammary carcinomas (Mackie et al., 1987*a*, Inaguma et al., 1988).

Little is known about the function of tenascin. Tenascin as a substratum supports the growth of primary mammary tumor cells in serum-free culture (Chiquet-Ehrismann et al.,

Dr. E. J. Mackie's present address is Sandoz AG, Preclinical Research 360/405, CH-4002 Basel, Switzerland.

1986) and promotes cartilage differentiation in vitro (Mackie et al., 1987*b*). Tenascin in the culture medium partially inhibits the attachment of wing-bud cells to fibronectin (Mackie et al., 1987*b*). In contrast, tenascin appears to act as a cell adhesion molecule in the central nervous system (Kruse et al., 1985).

This study was undertaken to investigate the distribution of tenascin in normal skin and healing skin wounds. The study revealed that tenascin is sparsely distributed in normal rat skin, but strongly expressed in healing wounds. The in vivo distribution studies, as well as in vitro studies, demonstrate that mechanisms regulating expression of tenascin are quite distinct from those for fibronectin.

Materials and Methods

Antisera

Tenascin was purified from the conditioned medium of primary rat embryo fibroblast cultures grown in DME containing 10% FCS. The purification was carried out according to the method described by Chiquet and Fambrough (1984*b*), but instead of an mAb column, a column containing polyclonal antiserum to chicken tenascin was used. Briefly, after ammonium sulphate precipitation and removal of fibronectin by gelatin-agarose (Sigma Chemical Co., St. Louis, MO), the extract was loaded onto a column containing rabbit antiserum to chicken tenascin (Chiquet-Ehrismann et al., 1986) coupled to cyanogen bromide–activated Sepharose-4B (Sigma Chemical Co.). The column was then washed with PBS and rat tenascin was eluted with 50 mM ethanolamine (pH 11.5) and then dialyzed against PBS. Antiserum to rat tenascin (~50 μg/injection) was produced in rabbits and processed as described for chicken tenascin (Chiquet-Ehrismann et al., 1986). The specificity of the antiserum was confirmed by Western blot analysis. The antiserum detected only two bands, of ~240 and 200 kD, in conditioned medium of rat embryo fibroblasts. This is in agreement with the subunit molecular weights previously described for rat tenascin (Chiquet-Ehrismann et al., 1986). The antiserum did not react with FCS or plasma fibronectin (Boehringer Mannheim, FRG).

Antiserum to mouse laminin was prepared in a guinea pig using the same procedure as for tenascin and 100 μg laminin (Bethesda Research Laboratories, Gaithersburg, MD) per injection. Rabbit antiserum to human plasma fibronectin was purchased from Gibco Laboratories (Basel, Switzerland).

Animals

3-mo-old male or female outbred Sprague-Dawley rats were anesthetized. The abdomen was shaved or depilated with a commercial depilatory cream and then three scalpel incisions, one deep, one medium, and one superficial, were made in the abdominal skin. Deep skin wounds were full-thickness wounds in which all layers were cut. Medium-thickness wounds cut through all layers except the cutaneous muscle and varying amounts of the adipose tissue layer. Superficial wounds were only cut through the epidermis and part of the dermis. Wounds were left uncovered and all healed without complications. Two animals were killed at each of the following time points: 15 and 24 h and 2, 3, 6, 10, 14, and 22 d. The wounds with some surrounding skin were excised and processed for histology. Four rats whose wounds were to be used for electron microscopy were also anesthetized 6 d after being wounded and then subjected to intracardiac perfusion with 4% paraformaldehyde and 0.1% glutaraldehyde in calcium- and magnesium-free Hanks' solution (CMF)[1] before the wounds were excised.

Histology

Tissues for immunohistochemistry at the light microscope level were fixed in 4% paraformaldehyde in potassium phosphate buffer (pH 7.4) for 4 h, washed in PBS, and then placed in 30% sucrose in PBS overnight. The wounds were then embedded in OCT compound (Tissue Tek; Miles Laboratories Inc., Naperville, IL), frozen rapidly on ethanol/dry ice, and stored at −70°C. Cryostat sections (10–20 μm) were cut and placed on gelatin-coated slides. Sections containing adipose tissue were placed in ether for 5 min to remove fat before staining. Sections to be stained by the immunoperoxidase method were then immersed in 0.3% hydrogen peroxide in 40% methanol to remove endogenous peroxidase staining. Some sections were treated with bovine testicular hyaluronidase (0.1 mg/ml at 22°C for 30 min; Calbiochem-Behring Corp., Lucerne, Switzerland) or type I collagenase (0.5 mg/ml) at 37°C for 20 min; Sigma Chemical Co.) before staining. Indirect immunofluorescence was carried out as described (Mackie et al., 1988). Some sections stained with anti-tenascin or preimmune serum, followed by FITC anti-rabbit Ig, were counterstained with TRITC-phalloidin (0.5 mg/ml in PBS for 2 min; Sigma Chemical Co.) and then washed in PBS before the coverslip was applied. Immunoperoxidase staining was carried out according to the same procedure but with peroxidase-labeled swine anti-rabbit or goat anti-guinea pig Ig (Dakopatts, Glostrup, Denmark) as the second antibody. Chloronaphthol was used as a chromogenic substrate.

Some sections were stained with hematoxylin and eosin by standard methods.

Electron Microscopy

The excised wounds were fixed for 2 h in the same solution used for perfusion (described above), washed several times in CMF and cryoprotected by immersion in 20% sucrose in CMF for 4 h. 60-μm free-floating sections were cut with a freezing microtome. Suppression of endogenous peroxidase activity was carried out as described above. After being rinsed several times, the sections were incubated with anti-tenascin or preimmune serum (1:100 in CMF containing 1 mg BSA/ml) for 2 h, washed (four times for 5 min each) in CMF, and then incubated in peroxidase-labeled goat anti-rabbit Ig for 2 h. The sections were washed for 1 h with several changes of buffer, and the peroxidase activity was developed with pyrocatechol/*p*-phenylenediamine (1 mg/ml in 0.15 M ammonium acetate buffer containing 0.01% hydrogen peroxide). The stained sections were washed, postfixed in 1% osmium tetroxide in cacodylate buffer, and embedded in EPON. The sections were resectioned and examined without counterstaining in a Philips electron microscope at 80 kV. Some wounds were fixed in 4% paraformaldehyde, 2.5% glutaraldehyde/CMF and processed for conventional EM histology.

Cell and Tissue Culture

Skin explants were cultured according to the method of Hashimoto and Marks (1984). Rats, as used for the wound experiments, were killed and the abdomens were depilated. Sheets of skin ~0.5 mm thick, were removed with a scalpel and cut into pieces ~3 × 2 mm. The explants were floated, epidermis upward, in DME with or without FCS. The explants were cultured for 1–7 d and then fixed and processed for histology as for the wound tissues.

Results

In this study, the distribution of tenascin in skin wounds of various thicknesses was compared with that of fibronectin. The results obtained for fibronectin in normal skin and healing wounds were in agreement with those previously obtained by Grinnell et al. (1981). The distribution of laminin was used to indicate the status of basement membranes. The distribution of tenascin was also compared with that of actin-containing wound fibroblasts, myofibroblasts, which were detected using TRITC-phalloidin, as previously described (Doillon et al., 1987).

Tenascin Is Associated with Basement Membranes in Normal Skin

In normal rat skin, tenascin was detected in a discontinuous distribution in the basement membrane of the dermal–epidermal junction, mostly close to hair follicles (Fig. 1 *B*). It was found in the basement membrane of subepidermal capillaries and hair follicles, but not of sebaceous glands, which are associated with hair follicles. There was strong tenascin staining in the sheaths of the large sensory tylotrich hair folli-

1. *Abbreviation used in this paper*: CMF, calcium- and magnesium-free Hank's solution.

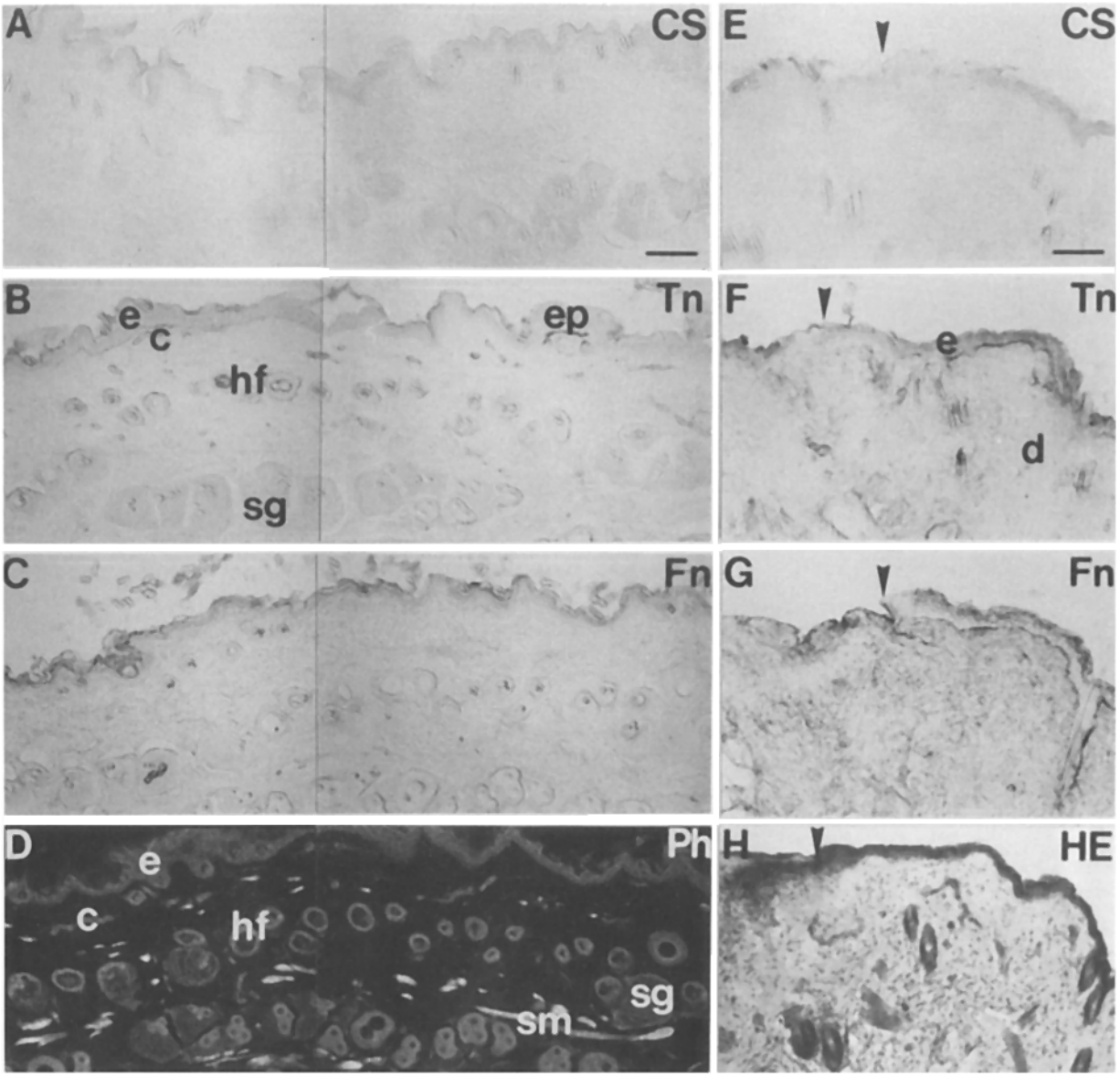


Figure 1. Distribution of tenascin in normal skin (*A–D*) and 24-h wounds (*E–H*). Hyaluronidase-treated sections stained by indirect immunoperoxidase with preimmune serum (CS; *A*, *E*), anti-tenascin (Tn; *B*, *F*), and anti-fibronectin (Fn; *C*, *G*). In *A–D*, oblique sections of hair follicles (hf) are seen at two levels, close to the surface of the skin, and deeper, where they are associated with sebaceous glands (sg). (*B*) In normal skin, tenascin is present discontinuously in the dermal–epidermal junction beneath the epidermis (e), but staining is strong beneath the epidermal pad (ep); it is also present in the basement membranes of dermal capillaries (c) and hair follicles, but not sebaceous glands. (*C*) Fibronectin is present in basement membranes and diffusely throughout the dermis. (*D*) Staining with TRITC-phalloidin (Ph) demonstrates that actin-containing fibroblasts are rare in the fibrous dermis. *sm*, smooth muscle. (*F*) 1 d after being wounded, tenascin staining is increased in the dermal–epidermal junction beneath the intact epidermis to the right of the cut edge of the wound (*arrow*). Tenascin staining can also now be seen diffusely in the dermis (d) adjacent to the wound. (*G*) Fibronectin staining is enhanced at all levels in the region of the wound. (*H*) Hematoxylin and eosin (HE) staining demonstrates the wound morphology. The dark band of cells to the left of the cut edge of the epithelium is an accumulation of polymorphonuclear leukocytes. Bar, 100 μm.

cles and of the epidermal pads, the area of thickened epidermis associated with tylotrich follicles (Straile, 1969). Otherwise, staining in the fibrous connective tissue of the dermis was undetectable, as was staining of dermal smooth muscle. Tenascin staining of normal skin was considerably enhanced by hyaluronidase treatment of sections but unaffected by collagenase treatment. Like tenascin, fibronectin was present in capillary, hair follicle, and dermal–epidermal junction basement membranes, but was also weakly and diffusely distributed throughout the fibrous tissue of the dermis (Fig. 1 *C*). TRITC-phalloidin showed faint, diffuse staining of the fibrous tissue of the normal dermis (Fig. 1 *d*), as previously described (Doillon et al., 1987).

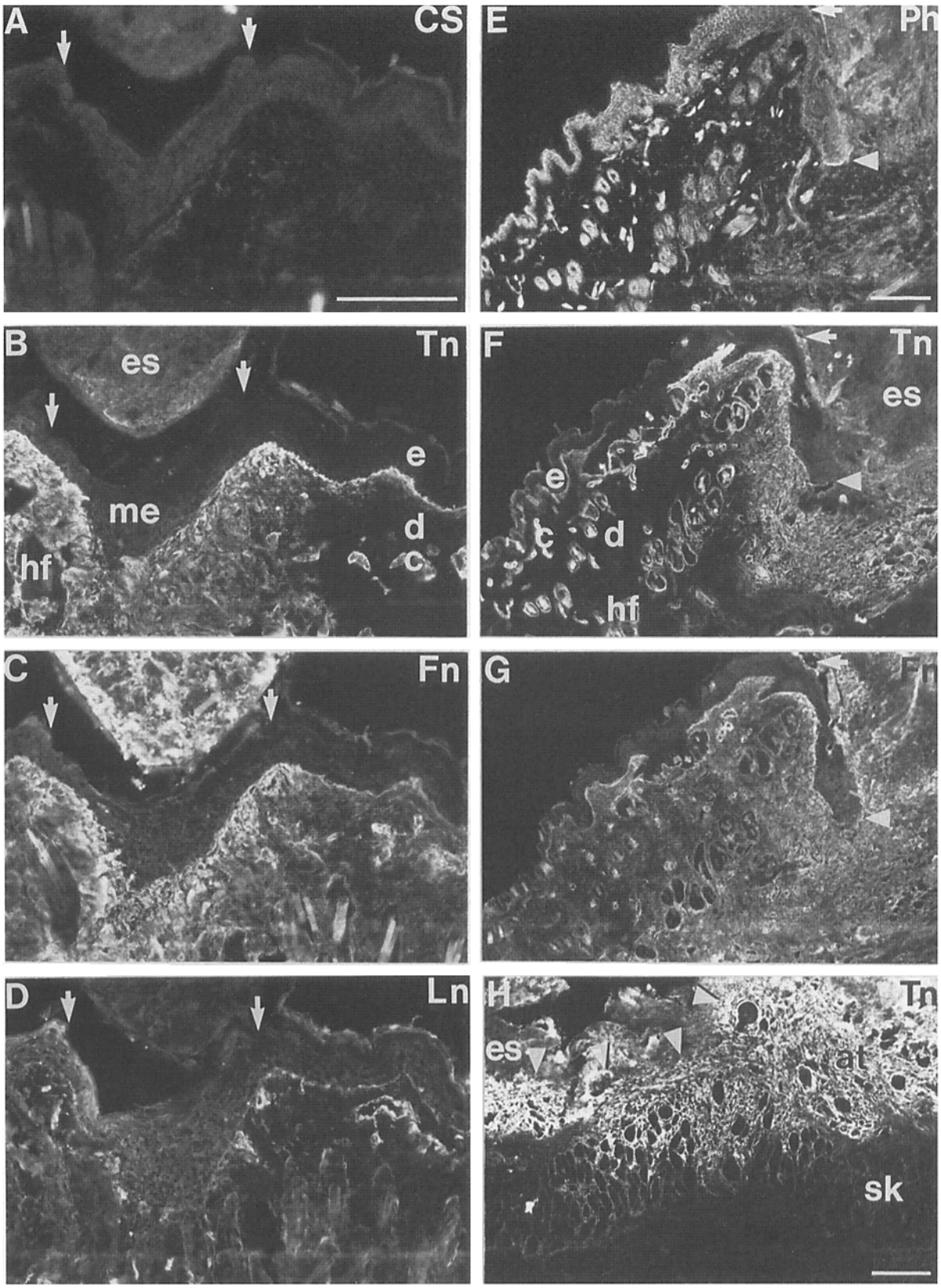


Figure 2. Distribution of extracellular matrix proteins in 3-d wounds. Fluorescence staining of adjacent hyaluronidase-treated sections of superficial (*A–D*), medium-thickness (*E–G*), and deep (*H*) wounds stained with preimmune (CS; *A*), anti-tenascin (Tn; *B, F,* and *H*), anti-fibronectin (Fn; *C, G*), or anti-laminin (Ln; *D*) serum or with TRITC-phalloidin (Ph; *E*). (*A–G*) Arrows indicate the point where the epidermis was cut. (*E–G*) Arrowheads indicate the point to which epidermis has migrated. (*A*) There is background fluorescence on the surface of the epidermis and in the eschar. (*B*) In a superficial wound, the wound edges separated slightly initially, but migrating epidermis (me) has already covered the wound space completely. Tenascin staining is very strong beneath the recently migrated epithelium

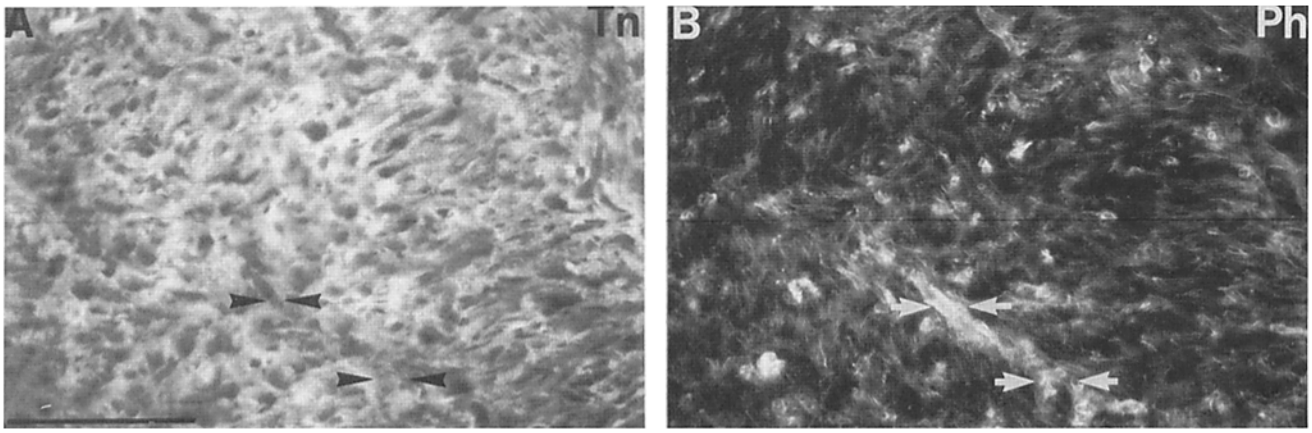


Figure 3. Distribution of tenascin in 6-d wounds. Fluorescence staining of a single section of granulation tissue in a deep wound with anti-tenascin (Tn; *A*) and TRITC-phalloidin (Ph; *B*). (*A*) Tenascin staining has a homogeneous extracellular distribution, whereas in the same field TRITC-phalloidin stains fine myofibroblast processes (*B*). The arrows outline a capillary. Bar, 100 μm.

Tenascin Is Strongly Expressed in Healing Wounds by Light Microscopy

After surgical incision, a blood clot forms within the wound. Polymorphonuclear leukocytes accumulate at the edges of the wound. Within ~24 h, epidermis at the edges of the wound thickens and begins to migrate between the dermis and the eschar, which is the remains of the clot and wound exudate. Over the next few days, the wound space is filled with granulation tissue, consisting mainly of fibroblasts, macrophages, and endothelial cells forming new blood vessels. Collagen fibers are deposited and gradually organized parallel to the wound surface; then wound contraction takes place. Wound myofibroblasts are thought to be important in the contraction process (Majno et al., 1971; Rungger-Braendle and Gabbiani, 1983). The time required for these events varies with the depth of the wound.

1 d after wounding, tenascin staining had become more intense at the dermal–epidermal junction and some staining was apparent in the dermis adjacent to the wound edge (Fig. 1 *f*). Fibronectin staining had become stronger in the dermis in the region surrounding the wound (Fig. 1 *G*).

3 d after wounding, epidermal migration across superficial wounds was complete. Tenascin was abundant at the dermal–epidermal junction underneath the newly migrated epithelium, and extending some distance from the wound edges towards the normal skin (Fig. 2 *B*). At this stage, laminin staining was not yet continuous at the dermal–epidermal junction (Fig. 2 *D*). In a 3-d medium-thickness wound, there was intense tenascin staining of the dermis at the wound edge, both in the region over which epidermis had already migrated, and beneath the eschar where epidermis had not yet migrated (Fig. 2 *F*). Epidermis thus was migrating over a surface rich in tenascin. In both superficial and medium-thickness wounds, the distinction between the wound area and adjacent normal tissue was much more obvious with tenascin staining (Fig. 2, *B* and *F*) than with fibronectin staining (Fig. 2, *C* and *G*). In a deeper 3-d wound in which the muscle layer was partially damaged, a sharp band of intense tenascin staining was visible all along the edge of the wound, in dermis, adipose tissue, and muscle layers (Fig. 2 *H*).

Because tenascin is known to be produced by fibroblasts in culture and by various mesenchymal tissues (see Introduction) it seemed likely that the specialized wound fibroblasts, myofibroblasts, produce tenascin. Double staining of sections with anti-tenascin and TRITC-phalloidin demonstrated that tenascin's distribution was not well correlated with that of myofibroblasts (Fig. 2, *E* and *F*). The area of dermis that was adjacent to the wound edge and contained myofibroblasts was more extensive than that containing tenascin.

In deep 6-d rat wounds, the wound space had been filled by a large amount of granulation tissue which had been partially covered by migrating epidermis. There was intense tenascin staining in the granulation tissue (Fig. 3 *A*). Newly formed blood vessels in the granulation tissue could be seen by phalloidin staining of endothelium (Fig. 3 *B*). Tenascin was not present in the basement membranes of these new vessels, which is demonstrated by comparison of tenascin and phalloidin staining of a single section at a high magnification (Fig. 3, *A* and *B*). This section also demonstrates that, although bundles of actin in myofibroblast processes could easily be observed (Fig. 3 *B*), very few tenascin-stained

and in the injured dermis. *c*, capillary; *d*, dermis; *e*, epithelium; *es*, eschar; *hf*, hair follicle. (*C*) Fibronectin staining is enhanced in the wound area, but is also present in the noninjured dermis. (*D*) Laminin staining beneath the newly migrated epidermis is not yet continuous. In a section of a medium-thickness wound, double-stained with TRITC-phalloidin (*E*) and anti-tenascin (*F*), the area of actin-containing myofibroblasts in the dermis is more widespread than the area of tenascin staining. (*F*) Tenascin is present in the dermis at the wound edge. Staining of the dermal–epidermal junction becomes weaker with increasing distance from the wound. (*G*) Fibronectin staining does not show the sharp distinction between wounded and nonwounded dermis that is seen with anti-tenascin (*F*). (*H*) Intense tenascin staining lines the edge of a deep wound in adipose tissue (at) and skeletal muscle (sk) layers. Arrowheads show the line between intact tissue and eschar. Bar, 100 μm.

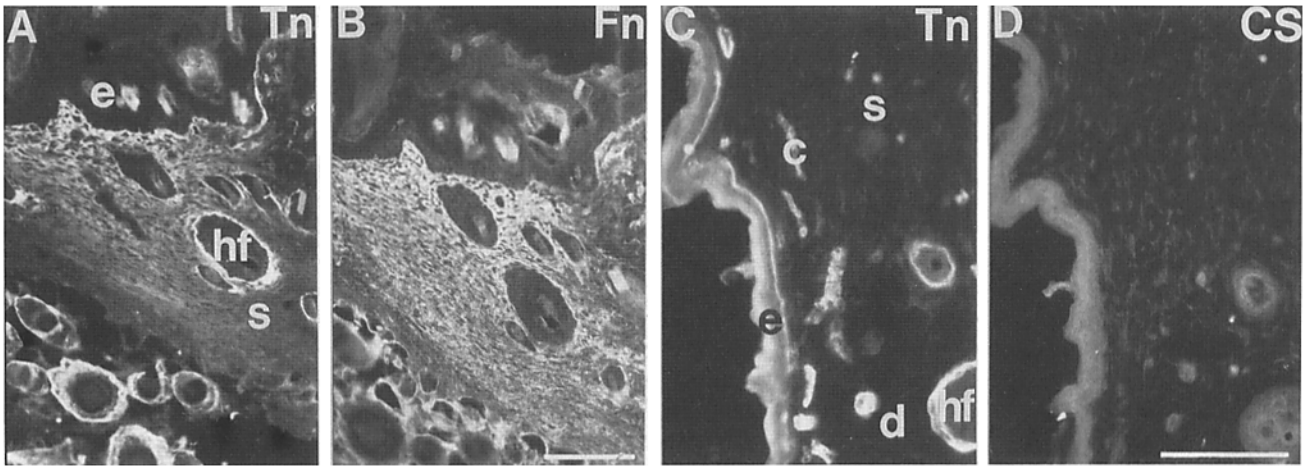


Figure 4. Distribution of tenascin in hyaluronidase-treated sections of wounds after contraction. (*A* and *B*) Fluorescence staining of adjacent sections of a 10-d medium-thickness wound with anti-tenascin (Tn; *A*) and anti-fibronectin (Fn; *B*). Tenascin is now almost absent deep in the scar (s), but remains at high levels high in the scar beneath the epidermis (e). *hf*, hair follicle. (*B*) Fibronectin is still abundant throughout the scar. (*C* and *D*) A deep 22-d wound stained with anti-tenascin (*C*) or preimmune (*D*) serum. (*C*) By 22 d, tenascin is found in capillary basement membranes within the scar, but is absent from the fibrous component of the scar. Bar, 100 μm.

fibrils were visible (Fig. 3 *A*). Tenascin thus was predominantly distributed homogeneously throughout the extracellular spaces of the granulation tissue at this stage.

With time, granulation tissue contracts, bringing the former wound edges back together. The abundant granulation tissue is replaced by a narrow scar of collagen fibers aligned parallel to the surface of the wound. Soon after contraction had taken place, in a medium-thickness wound at 10 d, tenascin was only just detectable deep in the scar, but still abundant high in the scar close to the dermal–epidermal junction (Fig. 4 *A*). In contrast, fibronectin was still evenly distributed in the scar (Fig. 4 *B*). Tenascin was not present in the basement membrane of blood vessels in the scar soon after contraction (Fig. 4 *A*), but had reappeared in the capillaries close to the epidermis some time later, when tenascin was totally undetectable in the fibrous tissue of the scar (Fig. 4 *C*).

Ultrastructure of the Dermal–Epidermal Junction in Normal and Wounded Skin

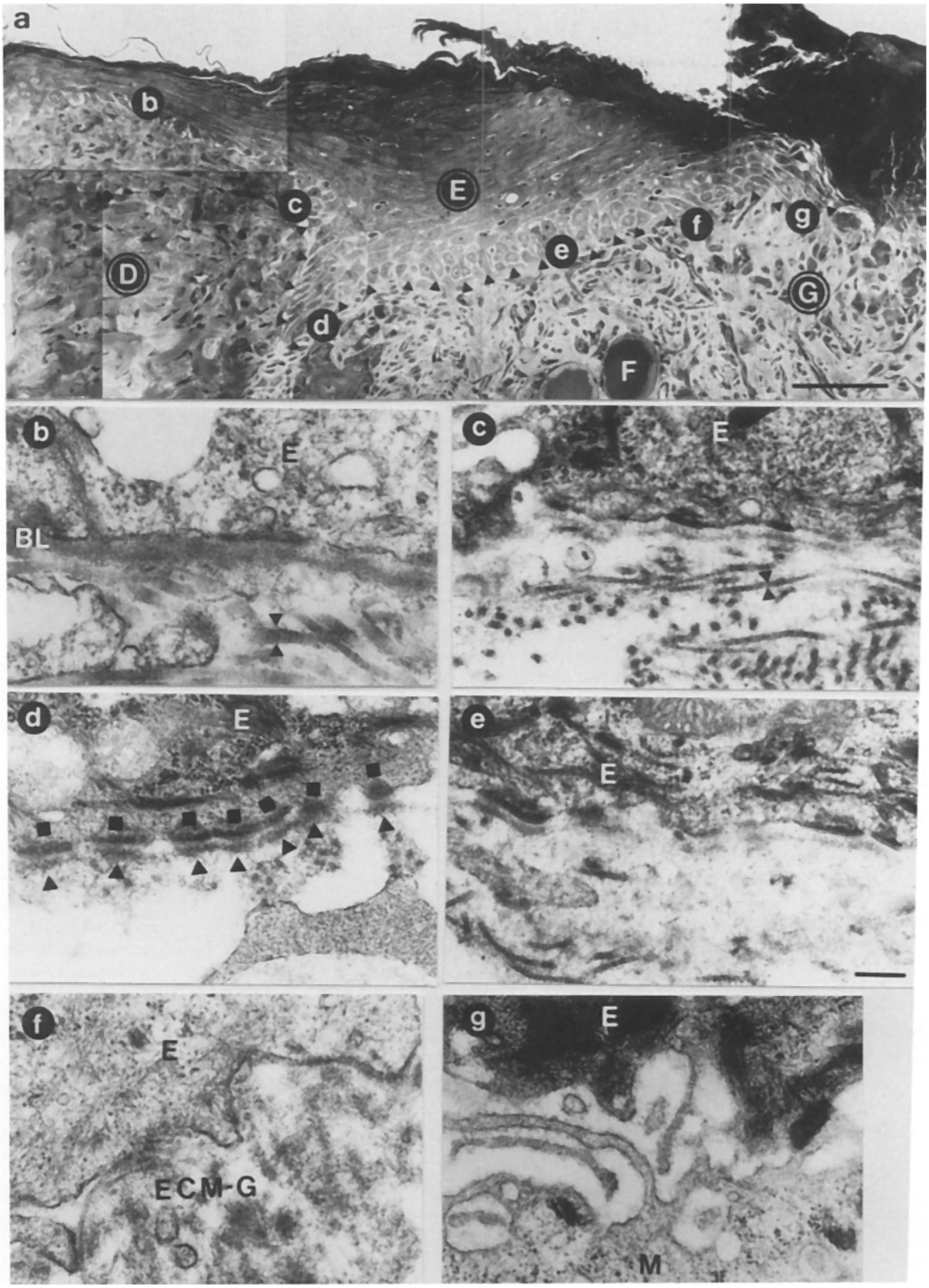
To understand in more detail the architecture of the dermal–epidermal junction, wound tissue with the adjacent unwounded skin was investigated by EM. Attention was paid to the reestablishment of the basal lamina between epidermis and dermis (Fig. 5). In normal skin, a 70-nm-thick basal lamina separated the epidermis from the dermis. The epidermal cells adhered to the basal lamina by numerous hemidesmosomes. The dermal stroma was invested with a dense network of 55-nm-thick collagen fibers (Fig. 5 *B*). From the margin to the center of the wound, the basal lamina became thinner (40 nm) and progressively more discontinuous (Fig. 5, *C–E*). The basal lamina fragments were always apposed to the epidermal hemidesmosomes (see Fig. 5 *D*). In the center of the wound, a basal lamina was not detectable and pioneer epidermal cells were sitting on an amorphous mass of extracellular matrix (Fig. 5 *F*), or were in contact with macrophages (Fig. 5 *G*). At the margin of the granulation tissue, the dermal stroma contained a network of 30-nm-thick collagen fibers (Fig. 5, *C–E*), whereas in the center of the wound amorphous matrix material was abundant (Fig. 5, *E* and *F*).

Ultrastructural Detection of Tenascin in Normal and Wound Tissue

In normal skin, tenascin was detected in the basal lamina of the dermal capillaries (Fig. 6 *A*) as well as in the basal lamina of the hair follicles (Fig. 7 *B*). The collagen fibers that were apposed to these basal laminae were also decorated with tenascin. Collagen fibers >2 μm away from the basal lamina were tenascin negative (Figs. 6 *A* and 7 *B*). The identity of the collagen fibers was established by counterstaining of the sections with uranyl acetate and lead citrate (Fig. 6 *B*).

In the wound tissue, the distribution of tenascin changed from the margin to the center of the wound. At the periphery, where epidermis was intact but thickened, tenascin antibodies stained both the basal lamina (predominantly apposing the hemidesmosomes) and the surface of the collagen fibers in the adjacent dermal stroma (Fig. 7, *C* and *D*). At the

Figure 5. (*A*) Semithin plastic section showing one half of a deep 6-d wound. At the left margin, normal epidermal cells (E) are resting on dermis (D), which consists of fibroblasts and collagen fibers. At the edge of the wound, there is an indentation of epidermis into dermis, as is often seen. At this point (*d*) there is a sharp division between normal dermis and granulation tissue (G). Note the different organization of normal dermis (few cells and many thick collagen fibers) and the granulation tissue (many cells and few or no collagen fibers). The border between epidermis and granulation tissue is indicated by arrowheads. *F*, fat. In *B–G*, EM photomicrographs show in more detail the various regions of the dermal–epidermal junction shown in (*A*). In normal skin (*B*), a thick basal lamina (BL), hemidesmosomes, and thick collagen fibers (*arrowheads*) are found. (*C*) At the margin of the wound the basal lamina is discontinuous and thinner. The collagen



fibers are also thinner (*arrowheads*: compare with collagen fibers in *B*). (*D* and *E*) The basal lamina fragments (*arrowheads*) are discontinuous and are found directly apposed to the epidermal hemidesmosomes (*squares*). (*F*) No basal lamina is present and amorphous extracellular matrix material (ECM-G) is present in the granulation tissue. (*g*) A pioneer epithelial cell contacts a macrophage (M). Bars: (*A*) 100 μm; (*B–G*) 0.25 μm.

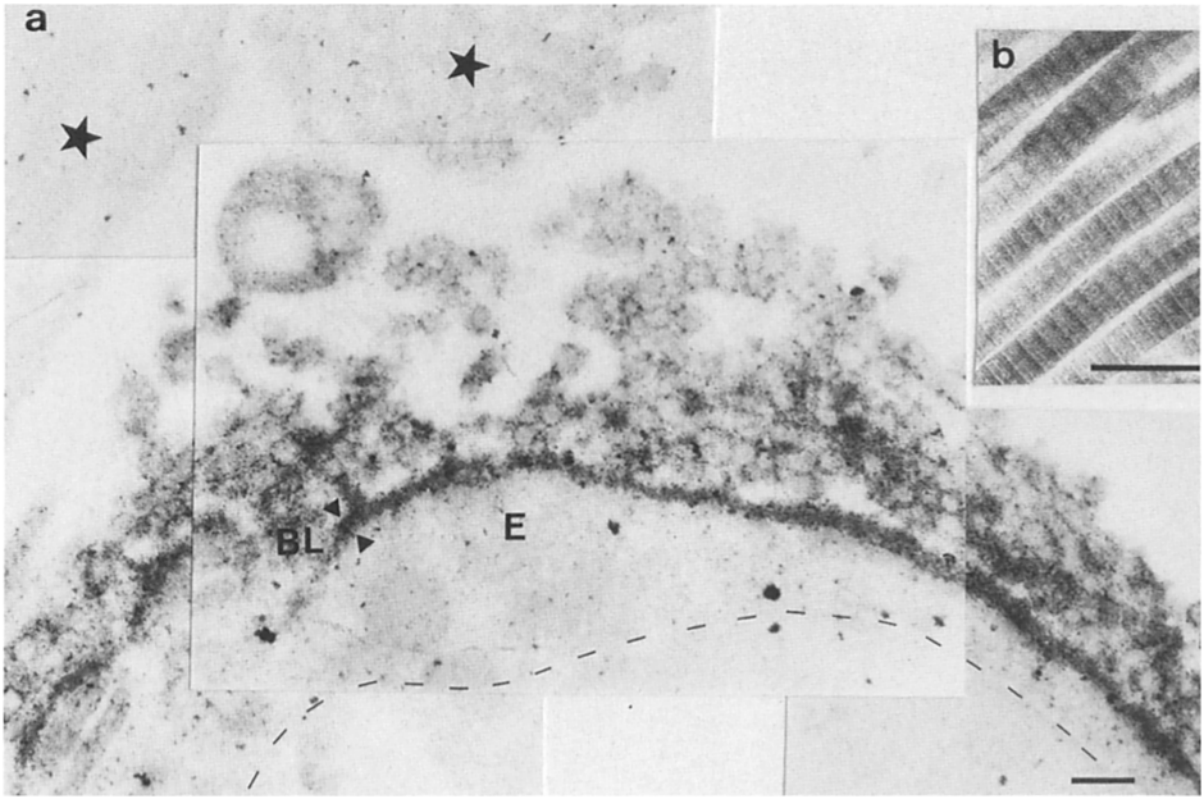


Figure 6. (*A*) Electron micrograph showing tenascin staining along the basal lamina (BL, *arrowheads*) of a dermal blood vessel in normal skin. Collagen fibers adjacent to the basal lamina are also stained; however, collagen fibers further away (*stars*) are negative. The collagen fibers were identified by the lead citrate and uranyl acetate staining of the same section (*B*). The luminal border of the endothelial cell (E) is indicated by a broken line. Bar, 0.25 μm.

former wound edge, where the earliest epidermal migration had occurred, tenascin was found in the basal lamina fragments that separated the epidermal from the dermal cells (Fig. 7 *E*). Staining of thick collagen fibers was no longer detectable and the tenascin in the stroma adjacent to the dermal–epidermal junction decorated an amorphous mass of extracellular matrix. In the central areas of the wound, tenascin was only detectable in the amorphous matrix of the granulation tissue (Fig. 7 *F*).

Migrating Epidermis Induces the Production of Tenascin by the Dermis

To determine whether any of the multitude of cytokines produced by the invading cells attracted to the site of injury were necessary for the production of tenascin by the dermis, skin explants were studied. It has previously been demonstrated that when skin explants are cultured floating on the surface of the medium, epidermis migrates over the cut edge of the dermis (Hashimoto et al., 1984). Epidermal cell migration even occurs, albeit more slowly, in serum-free medium.

When such skin explants were cultured and then sectioned and examined by histochemistry, it was found that tenascin staining was strong in the dermal–epidermal junction and adjacent dermis under migrating epithelium (Fig. 8 *B*). Epidermis from explants cultured in serum-free medium did not migrate as far, but tenascin was expressed under the epidermis that had migrated (not shown). Staining with anti-fibronectin (Fig. 7 *C*) and TRITC-phalloidin (Fig. 7 *D*) was weak and evenly spread throughout the sections, as in normal skin.

Discussion

Tenascin in normal skin was found to be only associated with basement membranes and hair follicle dermal sheaths. It was weakly and discontinuously detected at the dermal–epidermal junction. Tenascin was, however, strongly expressed in healing wounds. This dramatic change in expression suggests that tenascin is important in the wound healing process.

The association of tenascin with basement membranes, demonstrated in this study, is intriguing. Tenascin was detected in the basement membranes of subepidermal capillaries in normal skin, but not in capillaries of granulation tissue or the newly contracted scar. Tenascin was sparsely distributed in the basement membrane of normal epidermis, but abundant in the immature basement membrane of wound epidermis. In one situation tenascin thus indicates a mature basement membrane, and in another, an immature basement membrane.

Staining for tenascin was very intense at the dermal–epidermal junction beneath recently migrated epidermis and beneath the thickened, proliferating epidermis at the edges of the wound, extending towards the normal skin. In vitro, tenascin was again expressed at the dermal–epidermal junc-

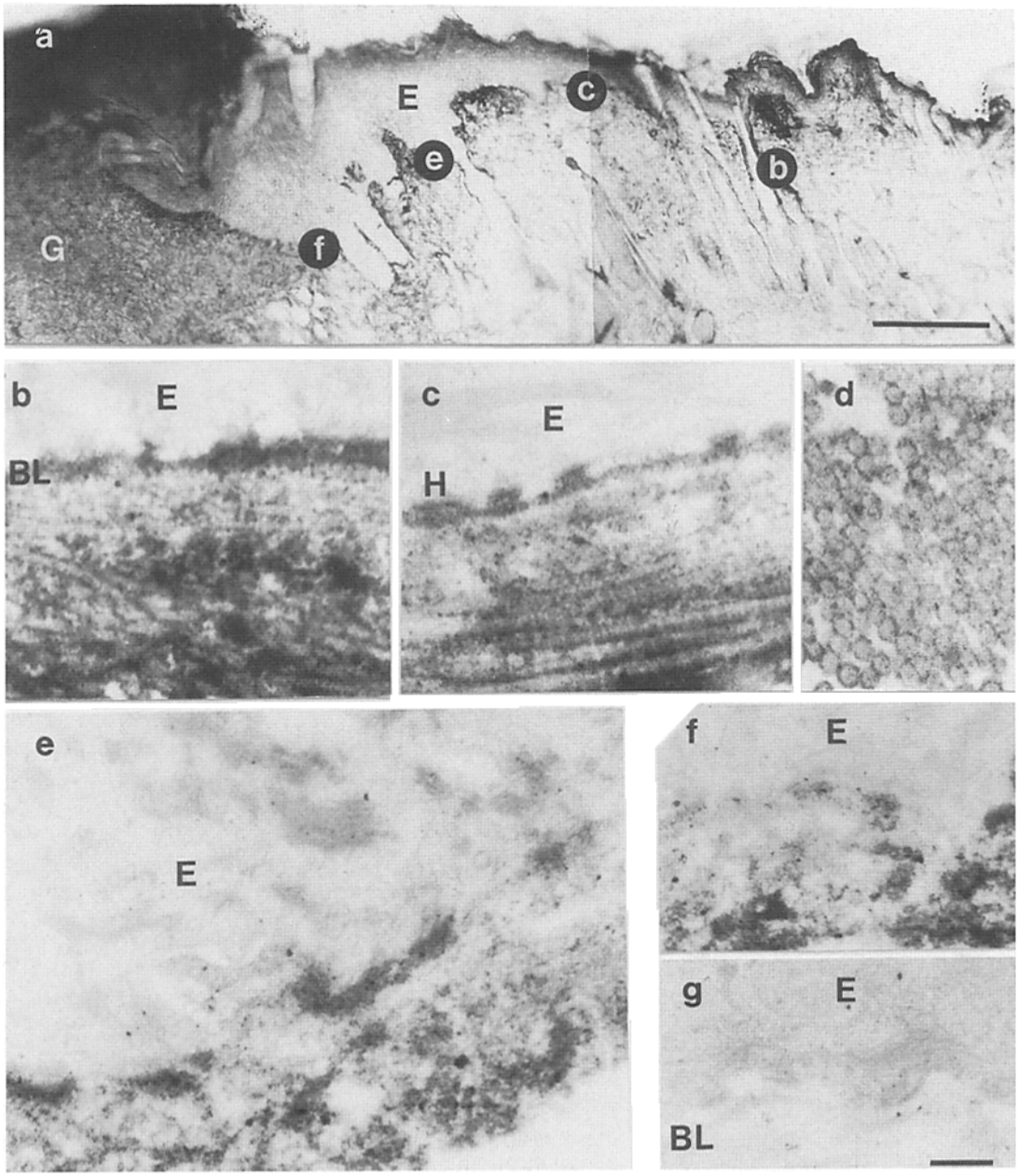


Figure 7. Immunohistochemical detection of tenascin in a wound and in adjacent skin. An overview of the tenascin distribution of a deep 6-d wound is shown in *A*. The center of the wound is on the left and unwounded skin is on the right. Tenascin staining was found in the basal lamina (BL) of hair follicles (*b*), of the dermal-epidermal junction at the wound edge (*C* and *E*) and in the granulation tissue (G). *E*, epidermis. The staining of the eschar and of the surface of the epidermis is not specific. In *B–F*, electron micrographs show the dermal-epidermal junction at high magnification. (*B*) In the hair follicle, tenascin is found in the basal lamina and on the surface of the collagen fibers in the adjacent dermal stroma. (*C*) At the wound edge, tenascin is found in the basal lamina between dermis and epidermis, predominantly apposed to the hemidesmosomes (H), and on the collagen fibers of the adjacent stroma. (*D*) Collagen fibers of the dermal stroma, decorated by anti-tenascin, are shown in cross section. (*E*) Further into the wound, anti-tenascin stains the discontinuous basal lamina fragments at the dermal-epidermal junction. In the stroma, tenascin stains the amorphous matrix of the granulation tissue. (*F*) Under recently migrated epidermis towards the center of the wound, tenascin is only found in matrix of the granulation tissue. No basal lamina is detectable. (*G*) No staining is seen in sections incubated with preimmune serum. Bars: (*A*) 100 μm; (*B–G*) 0.25 μm.

tion and in the dermis immediately underlying migrating epithelium. Such tenascin staining was not seen at the cut edge of the dermis, where epithelium had not yet migrated; it thus can be concluded that migrating, proliferating epithelium induces the expression of tenascin by dermal cells. This induction does not require the presence of serum or of the cell types, absent from normal skin, that appear in healing wounds.

Tenascin expressed at the dermal-epidermal junction may play a role in epidermal cell migration. Not only was tenascin found to be expressed in response to migrating epidermis, but it was also strongly expressed in the wound surface

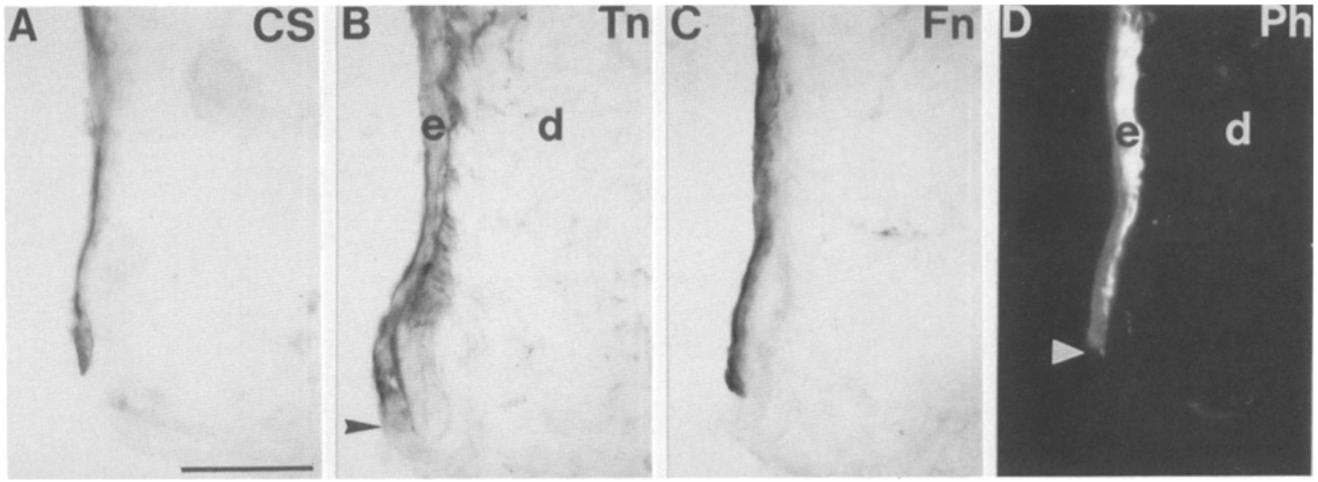


Figure 8. Induction of tenascin by migrating epithelium. Skin explants were cultured for 2 d in the presence of FCS, and then sectioned and stained by indirect immunoperoxidase with preimmune (CS; *A*), anti-tenascin (Tn; *B*) or anti-fibronectin (Fn; *C*) serum, or with TRITC-phalloidin (Ph; *D*). All the epidermis (e) visible in these micrographs has migrated over the cut edge of the dermis (d) during the culture period. The arrowhead denotes the front of migrating epidermis. (*B*) Strong tenascin staining is seen at the dermal-epidermal junction and in the adjacent dermis. Fibronectin (*C*) and actin-containing fibroblasts stained with TRITC-phalloidin (*D*) are distributed as in normal skin. Bar, 100 μm.

encountered by the front of migrating epidermis. The ability to migrate is an intrinsic property of epidermal cells, and can be markedly enhanced by epidermal growth factor and transforming growth factor-α (Barrandon and Green, 1987); tenascin thus is obviously not required for migration. It seems likely, however, that a tenascin-containing substratum could provide ideal conditions for optimal movement of an epidermal cell sheet across the surface of a wound. The distribution of tenascin is well correlated with the pathways of neural crest cell migration and it has been speculated that tenascin promotes the migration of neural crest cells by preventing them from attaching too well to fibronectin (Mackie et al., 1988). A similar mechanism could also be involved in epidermal cell migration. On the other hand, the finding that tenascin is localized in basement membrane fragments opposite hemidesmosomes suggests that tenascin may be involved in adhesion of migrating epidermis.

Tenascin was strongly expressed at wound edges in the dermis and in the granulation tissue that filled deep wounds. In these areas, the distribution of tenascin was not well correlated with that of myofibroblasts. The electron microscopic study demonstrates that, in the dermis, tenascin associates either directly or indirectly with native collagen fibers. The coating of collagen fibers with tenascin may alter the interaction of cells or other extracellular matrix components with collagen. In the young granulation tissue at the center of the wound, in which no collagen fibers were seen, tenascin was present in amorphous deposits. Perhaps tenascin is only ever present in a fibrillar distribution when it can associate with collagen fibers.

The presence of tenascin in granulation tissue suggests that it may play a role in the development of this transient tissue. Fibronectin and collagen are known to promote fibroblast migration (Knox et al., 1986; Postlethwaite et al., 1978). It is possible that tenascin, yet another extracellular matrix component, promotes migration into the wound area. The role of tenascin is, however, probably quite different from that of fibronectin, as their patterns of expression are so different. For example, tenascin may influence wound contraction. The contractile properties of myofibroblasts are widely thought to be responsible for wound contraction (reviewed by Rungger-Braendle and Gabbiani, 1983). It has been suggested, however, that because myofibroblasts remain in the scar for some time after contraction, it is unlikely that these cells alone account for wound contraction (Doillon et al., 1987). In this study, it was observed that granulation tissue ~5 mm across contracted to a scar ~50 μm wide. Whether or not myofibroblasts contract during wound contraction, they must also move considerably in relation to other cells and the extracellular matrix surrounding them for this 100-fold reduction in wound width to be able to occur. Indeed, Baur and Parks (1983) have proposed a model of wound contraction in which myofibroblasts use their contractile ability to migrate through the extracellular matrix. These authors have described the myofibroblast anchoring strand, a direct connection between actin bundles within the myofibroblast and collagen fibers without. This connection would allow the translation of migratory movement into tissue deformation or wound contraction. Tenascin, which inhibits the attachment of mesenchymal cells to fibronectin in vitro (Mackie et al., 1987*b*), could limit the myofibroblast's ability to adhere to the extracellular matrix surrounding it, thus allowing it to continue moving. Indeed, tenascin disappeared more rapidly than fibronectin or actin-containing myofibroblasts after wound contraction was complete. After contraction, unimpeded adherence of cells to the surrounding matrix through fibronectin would once more be desirable, to stabilize the scar.

The results of this study suggest that tenascin has an important and unique role in the wound healing process and demonstrate that its expression is subject to regulatory mechanisms quite different from those for fibronectin.

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