Fibronectin Distribution in Nailfold Biopsies of Scleroderma (Systemic Sclerosis) Patients

ZE-YI CHEN,1 HILDEGARD R. MARICQ,2 PATRICIA A. PILIA,3 RICHARD L. DOBSON,1 RICHARD M. SILVER2 and STERLING K. AINSWORTH3

1Department of Dermatology, Medical University of South Carolina, 2Department of Medicine, Medical University of South Carolina and 3Department of Pathology, Medical University of South Carolina, Charleston, South Carolina, USA


The distribution of fibronectin (FN) was studied in skin biopsies of 13 patients with scleroderma (SD), 7 patients with dermatomyositis (D), and 10 normal controls (NC) by direct immunofluorescence. In normal tissues, continuous or segmental linear staining of the dermal-epidermal junction (DEJ) was seen. Papillary, subpapillary dermis, and papillary capillary loops showed a reticular pattern of deposition with fibronectin. Scleroderma patients revealed similar staining in the dermis and DEJ. The reticular distribution of FN appeared to stain more intensely in the dermis than in controls, especially in deeper layers. The amount of FN in walls of blood vessels from SD patients was markedly increased; all dermal vessels stained with FN and revealed considerably thicker walls and larger lumens. FN distribution in DM patients was similar to that seen in SD with an increased amount of FN staining in capillary walls. Key words: Fibronectin, Nailfold biopsy; Scleroderma.

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S. K. Ainsworth, Department of Pathology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA.

Fibronectins (FN) are high molecular weight glycoproteins found both in plasma (previously known as cold insoluble globulin) and in various tissues associated with cell surfaces, basement membranes and extracellular connective tissue matrix components (1). A variety of cells, including fibroblasts, macrophages and endothelial cells, are able to synthesize FN in tissue culture (2-4). In normal skin, FN is found at the dermal-epidermal junction (DEJ), throughout the connective tissue of both papillary and reticular dermis, around skin appendages and in the blood vessel walls. The most intense staining by the immunofluorescence (IF) technique is seen in the capillaries of the papillary body (5). FN has not been reported in normal epidermis (5). The amount of FN in the skin has been shown to increase during processes involving tissue reorganization such as inflammation, tumor growth and wound repair (6-9). Both plasma-derived and newly synthesized FN seem involved, the former being related to increased capillary permeability and the latter to endothelial cell activation and capillary neoformation (7).

Injury to blood vessels has been proposed as a stimulus to FN production (10). Several publications have appeared in the literature studying FN in a variety of skin diseases that involve lesions of small blood vessels (6). Increased FN has also been found in pathological conditions that show increased fibrous tissue formations such as keloids (11). Scleroderma (SD) (systemic sclerosis) is a connective tissue disease of unknown origin in which both small blood vessel lesions and fibrosis are prominent. A few investigators have reported changes in FN distribution in scleroderma skin (12-14) agreeing that the largest departure from normal occurs in the deep dermis of the involved skin where the FN
localization appears to parallel the increase of collagen. In both involved and noninvolved SD skin, FN deposition in the papillary dermis is reported as similar to that of controls. Perivascular increase of FN in deep dermis is also mentioned (14), but departure from normal distribution of FN in small blood vessel has not been described.

In the present investigation, FN distribution in SD skin was studied in nailfold biopsies; striking capillary abnormalities have been observed by in vivo microscopy in this skin area (15).

MATERIALS AND METHODS

Subjects
Subjects included 13 patients with SD, 7 patients with dermatomyositis (DM) and 10 normal controls (NC). Patients with DM were included once they showed very similar nailfold capillary changes by in vivo capillary microscopy. No information on FN distribution in DM has appeared, to our knowledge, in the literature. The subjects' ages ranged from 27 to 65 (mean 45.0) in SD, from 17 to 66 (mean 35.7) in DM and from 20 to 50 (mean 32.5) in NC. The sample included 19 women (7 SD, 6 DM, and 6 NC) and 11 men (6 SD, 1 DM, and 4 NC). The duration of their disease ranged in years from 1 to 15 (mean 4.2) in SD and from 1 to 6 (mean 2.9) in DM. All SD patients had proximal skin involvement (including the trunk in 12).

Methods
Nailfold biopsy was performed as previously described (16): 3–5 mm punch biopsies were obtained from forearm and buttck on the same day in 10 of 13 SD patients and in all other study subjects for comparison with nailfold specimens. An informed consent form meeting the requirements of the Institutional Review Board for Human Research was signed by each participant.

Direct immunofluorescence
Direct immunofluorescence technique was used to determine the distribution of FN. The biopsies were embedded in O.C.T. compound (Lab-Tek Products, Davison, IL), frozen and stored at −70°C. Specimens were sectioned at 4 μm thickness with a cryostat (Harris, Billerica, MA). The sections were fixed with acetone for 10 min, air dried 15 min, washed twice for 10 min each with phosphate buffer saline (PBS, pH 7.2) and treated with rabbit antihuman fibronectin conjugated with fluorescein isothiocyanate (FITC, E.Y. Laboratories, San Mateo, CA). Protein concentration of antibody preparation was 5 mg/ml, with a molar F.P. ratio of 1.5. Conjugate was diluted 1:10 with PBS. Sections were overlayed with conjugated antibody preparation and incubated in a moist chamber for 30 min. The slides were washed twice for 15 min in PBS with magnetic stirring, and coverslipped with glycerine in PBS. The tissue sections were read with a Zeiss Universal Fluorescent microscope with a BP filter 490/20/0.9. Photographs were taken with Kodak Ektachrome 400 film.

The control stainings were prepared by preincubation of sections with unlabelled goat anti-human fibronectin antibody preparation (Cappel Laboratories, Inc., Dowington, PA), followed by staining with FITC-conjugated anti-fibronectin antibody.

RESULTS

Nailfold biopsies
Normal subjects. The distribution of FN in nailfold biopsies of normal control subjects was similar to that previously reported for normal skin (5). Continuous or segmental linear staining was present at the DEJ. The papillary and subpapillary dermis showed a reticular pattern of FN in all subjects. Papillary capillary loops were clearly stained by this technique in all specimens and showed a stronger fluorescence on the dorsal side of the nailfold compared to the ventral side (Figs. 1a and 2a).

SD patients. Observations in epidermis and DEJ were similar to those seen in normal subjects. The reticular distribution of FN in the dermis had a more intense fluorescence than seen in controls, especially in the deeper layers. In two patients, a heavy FN accumulation was found in the apex of the nailfold (Fig. 3a) and was associated with
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Fig. 1a. Direct immunofluorescence of nailfold biopsies from normal control subjects revealed a reticular staining pattern for fibronectin in the papillary and subpapillary dermis. Capillary loops demonstrated a heavy fibronectin localization on the dorsal side of the nailfold (arrows) compared to the ventral side (see Fig. 2a). (FITC-antihuman fibronectin: ×160.)

Fig. 1b. Direct immunofluorescent staining of nailfold biopsies from scleroderma patients revealed a heavier reticular deposition of fibronectin in the dermis than seen in controls. Dermal vessels appeared dilated and outlined by heavy fibronectin staining. (FITC-antihuman fibronectin: ×165.)

Fibrinogen deposits (Fig. 3b). The most striking difference from normal controls was the amount of FN in the blood vessel walls of scleroderma patients. All dermal vessels were outlined by FN staining and showed considerably thicker walls than in normal control specimens by an estimated factor of 2 to 3 times, although not all capillaries of each patient were changed to such an extent. Capillary lumens were also considerably larger in SD patients (Fig. 1b) compared to normal controls (Fig. 1a).

DM patients. FN distribution in DM patients was similar to that seen in SD and the increased amount of FN in capillary walls was again the primary difference with normal subjects (Figs. 2a and 2b).

Biopsies from forearm and buttocks

As previously reported (12–14), there was no difference in FN distribution between normal control skin and non-involved skin of SD patients. Biopsy specimens from the involved

Fig. 2a. Direct immunofluorescence of nailfold biopsies show light fibronectin staining of ventral nailfold capillaries of a normal subject (arrows). (FITC-antihuman fibronectin: ×230.)

Fig. 2b. Direct immunofluorescence of a ventral nailfold biopsy from a DM patient reveals heavy staining of capillaries (arrows). (FITC-antihuman fibronectin: ×450.)
Fig. 3a. Direct immunofluorescent examination of nailfold biopsies from SD patients revealed a heavy accumulation of fibronectin in the apex. (FITC-antihuman fibronectin: x 190.)

Fig. 3b. Direct immunofluorescent examination of nailfold biopsies from SD patients revealed that the heavy apex deposition of fibronectin was associated with heavy fibrinogen staining. (FITC-antihuman fibrinogen: x180.)

skin showed increased fluorescence in the deep dermis, again as previously shown (12-14). Biopsy specimens from patients with DM were all from non-involved skin and could not be differentiated from normal controls.

DISCUSSION

In this study, the specimens from conventional biopsy sites, such as the forearm and buttock, confirmed earlier reports of increased FN accumulation in deep dermis of involved skin. Nailfold biopsies on the other hand revealed additional differences from normal controls, i.e. increased amounts of FN associated with the capillary wall. The direct immunofluorescent technique employed in this study, does not enable us to define the exact location of the FN within or around the capillary wall, nor the origin of this FN. However, based on the work of investigators studying inflammation and wound repair (7-10), one may speculate that both plasma-derived and newly synthesized FN may be present in the SD nailfold. Increased capillary permeability of nailfold capillaries in SD has been suggested by several earlier studies (17, 18). The FN associated with fibrinogen in the apex portion of the nailfold could thus be considered as plasma-derived and may reflect a considerable increase in capillary leakage and/or disruption of capillary wall. On the other hand, several in vivo observations of the nailfold capillary bed in SD and DM have shown considerable reorganization of this microvascular bed over time and "bushy" branchy capillaries, suggesting capillary neoformation (15, 19, 20); therefore, FN associated with capillary walls may be at least partly due to new synthesis, possibly by endothelial cells. Findings of increased FN associated with capillary walls in the nailfold of patients who show characteristic capillary abnormalities by in vivo microscopy support the hypothesis that an active process is operating at the microvascular level in this region of the skin and that capillaroscopic observations do not show the final end stage of fibrosis and capillary loss in these patients. It is hoped that the development of a more quantitative technique
may enable correlation of the pathological processes with other clinical and laboratory changes occurring in these patients.

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