Immunohistochemistry of Elastotic Material in Sun-Damaged Skin

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The nature of elastotic material in sun-damaged human skin was investigated by indirect immunofluorescence. Antibodies were used against the following components of the dermis: type I and type VI collagens, aminopropeptide of type I and type III procollagens, fibronectin, elastin, microfibrillar proteins, and basement membrane represented by the 7S domain of type IV collagen, laminin, and nidogen. The elastotic material exhibited marked fluorescence for elastin and microfibrillar proteins which codistributed with fibronectin. The presence of type I and VI collagens and procollagen type III were demonstrated to a lesser extent within the elastotic material. These results suggest that solar elastosis is primarily derived from elastic fibers and not from preexisting or newly synthesized collagens. J Invest Dermatol 87:334–337, 1986

MATERIALS AND METHODS

Tissue Biopsies of severely actinically damaged skin were obtained under local anesthesia from the posterior neck (cutis rhomboidalis) of 3 patients.

Histology Tissues were fixed in buffered formalin and stained with hematoxylin and eosin, Van Gieson's and Verhoeff's elastic tissue.

Preparation of Antibodies Affinity-purified antibodies against type I collagen and the aminopropeptide of procollagens I and III of bovine origin were prepared as previously described [7]. Antibodies against type VI collagen were raised against a pepsin fragment from human placenta [8] and those against fibronectin were prepared with material obtained from human plasma [9]. Antibodies against laminin [10], nidogen [11], and the 7S-domain of collagen IV [12] were prepared from basement membranes derived from the mouse Engelbreth-Holm-Swarm (EHS) tumor. Antibodies against human aortic alpha-elastin were obtained from Elastin Products Co. (Pacific, Missouri) while antibodies against microfibrillar proteins were prepared as previously described [13]. Those antibodies raised with antigens of nonhuman origin showed enough cross-reactivity with human tissue as previously shown [14].

Indirect Immunofluorescence Microscopy Indirect immunofluorescence microscopy was carried out as previously reported [15]. Briefly, frozen sections about 6–8 μm thick were placed in acetone for 5 min, washed in phosphate-buffered saline (PBS), pH 7.2, and digested for 30 min with hyaluronidase (2 mg/ml) in PBS, pH 5. Sections were treated for 30 min with the specific rabbit antibody, washed in PBS, and then stained for 45 min with goat antirabbit γ globulin antibodies conjugated with fluorescein isothiocyanate. The specimens were examined in a Nikon microscope equipped for epifluorescence overhead immunofluorescence. Photographs were taken with Kodak Ektachrome ASA 400 daylight film.

RESULTS

Biopsies of severely sun-damaged skin from the posterior neck of 3 patients revealed similar findings by light microscopy and indirect immunofluorescence. Verhoeff-van Gieson stained sec-
Figure 1. Indirect immunofluorescence of human actinally damaged skin. a, Verhoeff's-van Gieson stain (× 160). b, Antibody directed against type I collagen showing amorphous elastotic material (EL) in the upper dermis (D) separated from the epidermis (E) by collagen (× 160). c, As in (b) except at higher magnification (× 560). Note that remnants of collagen (arrowhead) bundles are visualized within the elastotic material (arrow) but that the elastotic material itself does not stain.

Figure 2. Immunofluorescence staining of elastotic material for the aminopropeptide of type III collagen (a, × 160; b, × 560). Staining for the aminopropeptide of type III collagen (arrowhead) is seen within the elastotic area. E = Epidermis, D = uninvolved portion of the dermis, EL = elastotic area, arrow = elastotic material.

Figure 3. Immunofluorescence staining of elastotic area for type VI collagen (× 160). Note staining of elastotic area (EL) while papillary layer of the dermis (D) is negative. E = Epidermis.
the dermis. In contrast, elastotic material showed intense staining in the form of large amorphous masses or thick fibers. These observations correlate with previous electron microscopic studies of sun-exposed skin showing an increase in elastic fibers and microfibrils [17]. Similar changes have also been demonstrated in hairless mice exposed to UV radiation [3]. Staining for the interstitial collagens (types I and VI collagen and the aminopropeptide of type III collagen) suggest their presence within the elastotic material. Fine collagen fibrils within the elastotic material [3] may represent immature type I collagen or mature type III collagen, since the latter collagen grows only to about 30-40 nm. The finding of type VI collagen arranged in a fine network may suggest a possible interaction with microfibrils.

The association of fibronectin with the elastotic material suggests that either it interacts with altered elastic tissue or that it is a major constituent of solar elastosis. Immunoelectron microscopy [18] and biochemical studies [13] support the interaction of fibronectin with normal elastic fibers, particularly microfibrils. Similar interactions of fibronectin and elastic tissue were noted in pseudoxanthoma elasticum [19].

No changes in the basement membrane components (type IV collagen, laminin, and nidogen) were detected around blood vessels, appendages, or along the dermal-epidermal junction of actinically damaged skin. Nidogen is a recently discovered glycoprotein of basement membranes which codistributes with laminin and type IV collagen [11]. Thickened capillary basement membranes have been observed by periodic acid-Schiff staining as well as electron microscopy in previous studies of sun-damaged skin [3,17]. In this study, the vessels within the elastotic material appear to have patent lumens and walls of normal thickness. However, it is very difficult to assess wall thickness of small blood vessels by immunofluorescence microscopy.

In summary, this study demonstrates that the elastotic material of sun-damaged human skin is composed primarily of elastic fibers intermingled with type VI collagen and remnants of type I and type III collagen fibrils. Fibronectin appears to interact with the elastotic material. Biochemical analysis of this elastotic material is currently in progress to further characterize its composition.

REFERENCES


