Immunochemistry 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*

Cumulative, repeated exposures to natural sunlight or artificial ultraviolet radiation (UVA-UVB) contribute to the development of degenerative alterations of the skin, clinically characterized by wrinkling, atrophy, dryness, telangiectases, and pigmentary changes. The posterior neck is commonly affected and can become furrowed, thickened, and yellowish. This condition is known as cutis rhomboidalis nuchae, "farmer's skin," or "sailor's skin." The major histologic finding of actinically damaged skin is the presence of basophilic fibers in the upper dermis, referred to as elastotic material, or solar elastosis [1,2]. In experimental animals, exposure to UV radiation results in an increase of elastic tissue in a fibrillar pattern [3,4].

Despite numerous clinical, pathologic, and biochemical studies, very little is understood about the biochemical composition of solar elastosis. Some investigators have suggested altered elastin to be the primary component of elastotic material, because it has similar histochemical staining reactions to elastic tissue. Biochemical analysis has shown the amino acid composition of elastotic material to be similar to normal human elastin, with lower hydroxyproline and proline contents than collagen [5]. In addition, elastotic material is susceptible to degradation by elastase but not collagenase [1]. Other investigators, however, believe that elastotic material represents altered collagen which merely acquires the tinctorial properties of elastin [6]. This study was conducted to identify the constituents of the elastotic material in actinically damaged human skin by indirect immunofluorescence microscopy using antibodies directed against various collagens, elastic tissue components, fibronectin, and components of the basement membrane.

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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-

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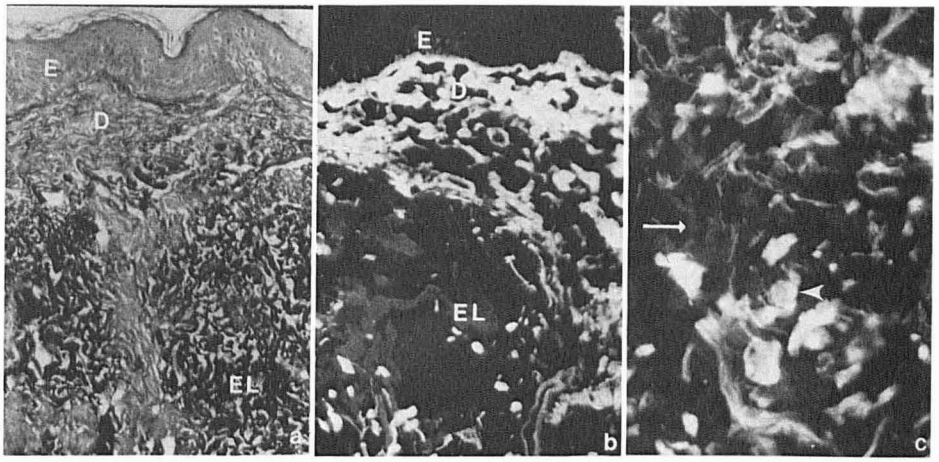

Figure 1. Indirect immunofluorescence of human actinically damaged skin. *a*, Verhoeff's-van Gieson stain (× 160). *b*, Antibody directed against type I collagen showing amorphous elastotic material (*EL*) in the upper dermis (*D*) separated from the epidermis (*E*) by collagen (× 160). *c*, As in (*b*) 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.

tions (Fig 1*a*) revealed thickened, spaghetti-like black fibers occupying the papillary dermis and upper reticular dermis with sparing of the superficial papillary dermis. These fibers stained positively for elastic tissue. Indirect immunofluorescence using antibody directed against type I collagen left large unstained areas corresponding to elastotic material, which were surrounded by thick collagen bundles (Fig 1*b*). However, at higher magnification, remnants of type I collagen in the form of thin fibrils or large clumps were seen within the area of elastotic material (Fig 1*c*). Staining for the aminopropeptide of type I collagen was limited to the dermal-epidermal junction, a finding similar to that seen in normal human skin as previously shown [7,15], with no detectable fluorescence in the elastotic material. Staining for the aminopropeptide of type III collagen (Fig 2*a*) revealed a network of fibers throughout the area of elastotic material (Fig 2*b*). Type VI collagen was observed with faint fluorescence in the form of a fine network within the affected area (Fig 3). The elastotic material was found to stain intensely in a fibrillar pattern when antibody directed against elastin was used (Fig 4*a*). This pattern contrasts with the faint reticular pattern observed in uninvolved dermis (not shown). Similarly, increased staining of microfibrillar proteins was detected in the elastotic material relative to the lower unaffected dermis (Fig 4*b*). Staining for fibronectin revealed marked fluorescence throughout the elastotic material (Fig 5). Basement membrane proteins (type IV collagen, laminin, and nidogen) were found in a pattern similar to that of normal skin and were localized around blood vessels, appendages, and along the dermal-epidermal junction. A network of patent capillaries was present within the elastotic material (Fig 6). No significant background staining was observed with IgG from nonimmunized rabbits (Fig 4*c*).

DISCUSSION

In this study, we have demonstrated by indirect immunofluorescence the distribution of various dermal components in elastotic material. This material was found to consist predominantly of elastin, microfibrillar proteins, and fibronectin, with the interstitial collagens present to a lesser extent. These findings confirm previous observations suggesting that elastotic material is primarily derived from elastic fibers.

Elastic fibers consist of an amorphous component known as elastin and microfibrils which may be found within and at the periphery of elastin. During development, microfibrils are arranged together to act as a scaffolding upon which elastin is deposited [16]. Normal human skin stained for either microfibrils or elastin demonstrated a fibrillar or reticular pattern throughout

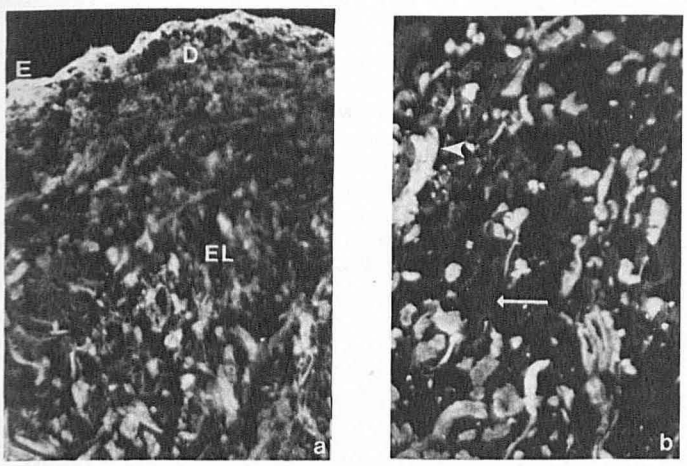

Figure 2. Immunofluorescence staining of elastotic material for the aminopropeptide of type III collagen (*a*, × 160; *b*, × 560). Staining for the aminopropeptide of type III collagen (*arrowhead*) is seen within the elastotic area. *E* = Epidermis, *D* = uninvolved portion of the dermis, *EL* = elastotic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

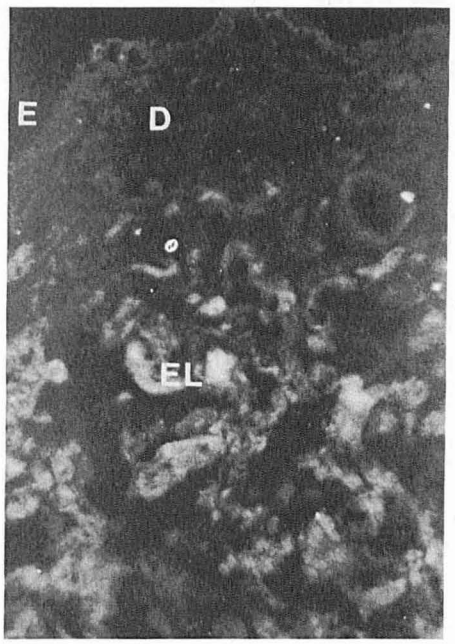

Figure 3. Immunofluorescence staining of elastotic area for type VI collagen (× 160). Note staining of elastotic area (*EL*) while papillary layer of the dermis (*D*) is negative. *E* = Epidermis.

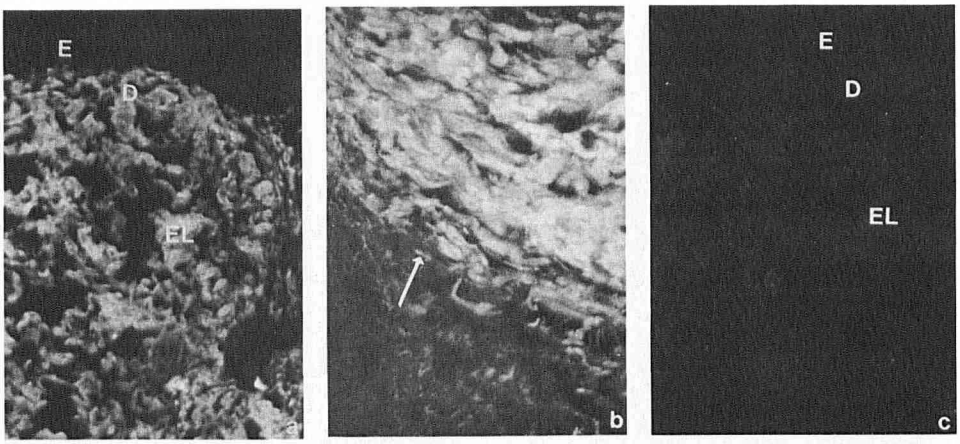

Figure 4. Indirect immunofluorescence of elastotic area. *a*, Incubated with antibodies to elastin (× 160). *b*, Incubated with antibodies to microfibrillar proteins (× 560). Note the sharp line of demarcation (*arrow*) between the lower edge of the elastotic material and the lower reticular dermis. *c*, Control section incubated with preimmune sera (× 160). *E* = Epidermis, *D* = uninvolved portion of the dermis, *EL* = elastotic area.

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].

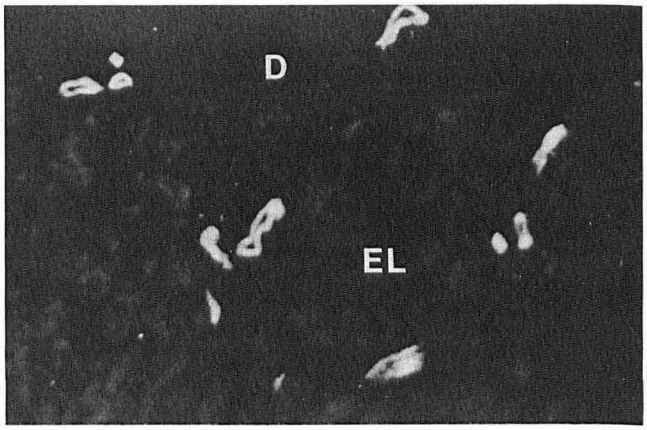

Figure 6. Immunofluorescence staining of elastotic area for type IV collagen (× 560). Note capillaries with patent lumens and normal walls. *D* = Uninvolved portion of the dermis, *EL* = elastotic area.

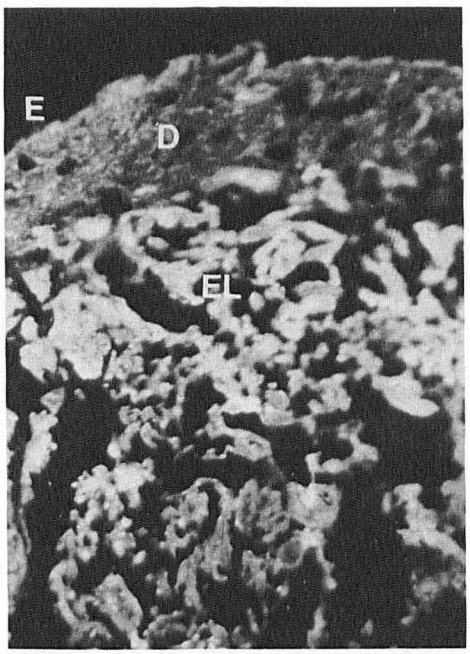

Figure 5. Immunofluorescence staining of elastotic area for fibronectin (× 160). Note that the papillary layer consisting mostly of collagen stains faintly. *E* = Epidermis, *D* = uninvolved portion of the dermis, *EL* = elastotic area.

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.

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