Immunofluorescent Localization of Type I and III Collagens in Normal Human Skin with Polyclonal and Monoclonal Antibodies

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Anti-type I and type III collagen polyclonal antibodies and anti-type III collagen monoclonal antibodies were produced using type I and type III collagen extracted from the human placenta. An indirect immunofluorescence technique using these antibodies showed the same distribution of type III as well as type I collagen in the entire dermis of the normal human skin in nearly identical patterns. Previous immunofluorescent study indicated that type III collagen is present predominantly in the papillary dermis. However, our observation that monoclonal antibody recognizing the helical portion of type III collagen reacted with the entire thickness of the dermis which suggested the presence of type I and III collagen in close proximity in the whole thickness of normal human dermis. (Received July 8, 1987.)

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Type I and III collagens are quantitatively predominant components of collagens of the normal human skin. A decade ago, Meigel et al. suggested immunohistologically that type I collagen was found in all dermal layers and that type III collagen could be found mainly within the adventitial dermis (1). Immunoelectron microscopic study suggested that type III collagen was found around small blood vessels, adipocytes and smooth muscle cells (2). However, contradictory results were found by biochemical analysis (3, 4, 5, 6). Clarification of the distribution patterns of collagens and their relationships is considered to be important in elucidating their physiologic and pathologic roles in skin. Morphological approaches with more convincing evidence have not been reported to our knowledge. Previous immunohistological studies employed only polyclonal antibodies. In this study, we used both a monoclonal antibody to type III collagen and polyclonal antibodies for indirect immunofluorescence analysis. The aim of this study is to reexamine the immunofluorescent staining patterns of these collagens.

MATERIALS AND METHOD

Skin specimens

Skin specimens were obtained from a total of 36 exposed and covered areas of 16 subjects of both sexes aged two to 85 years. Each specimen was embedded directly in O.C.T. compound, snap-frozen in liquid nitrogen, and stored at −80°C.

Antibodies

Collagens (types I, III, IV, V and VI) were solubilized and extracted from the human placenta by pepsin digestion (7, 8, 9). Type II collagen was extracted from newborn costal cartilage of a human

autopsy case according to the method of Miller & Matukas (10). Rabbits were immunized by subcutaneous injection of purified collagen type I or type III (10 mg/rabbit) emulsified with an equal volume of complete Freund's adjuvant. Injection was further repeated five times at two week intervals. Antibodies thus obtained were purified by collagen-coupled affinity chromatography, as described (11). Specificity of antibodies were determined by an enzyme-linked immunosorbent assay using microtitration plate coated with type I, II, III, IV, V and VI collagens, laminin, and fibronectin (12). The antibody reacted with antigen used for immunization. Monoclonal antibody against type III collagen was prepared as previously described (13).

Immunofluorescence

Four micron acetone-fixed cryostat sections were stained by immunofluorescence using these monoclonal and polyclonal antibodies. Anti-type I and III collagen antisera were used at 1:40 dilution and monoclonal antibody against type III collagen was used at a protein concentration of 3.76 μg/ml (1:1000 dilution ascites fluid). Sections were treated for 15 min at room temperature with the primary antibodies. After washing three times in PBS, the sections were incubated with fluoresceinated affinity purified goat $F(ab')_2$ anti-rabbit IgG (TAGO) or goat $F(ab')_2$ anti-mouse IgG (TAGO) for 30 min at room temperature. After washing three times in PBS, sections were mounted in 50% glycerine in PBS and examined using a Nikon VFD-R fluorescence microscope. In control experiments, specific antibodies were replaced by normal rabbit serum, normal mouse serum, or PBS.

RESULTS

In the controls, yellow autofluorescence of elastic fibers alone was observed in the dermis (Fig. 1). Anti-type I collagen antibody reacted with the entire thickness of the dermis (Fig. 2). A reaction was also found around the eccrine glands and apocrine glands (Figs. 3 and 4). Anti-type III collagen monoclonal antibody reacted with the entire dermis diffusely (Fig. 5). A reaction was also observed in the thick collagen bundles in the reticular dermis. It was also found around the eccrine glands and apocrine glands but not around the ductal portion of the eccrine glands (Figs. 6, 7 and 8). The interior of the arrector pili was positive showing a reticular pattern (Fig. 9). An identical result was obtained with polyclonal antibody against type III collagen (Fig. 10). The distribution patterns of the reaction of both anti-type I and type III antibody were not related to sex, age, or whether the sample was taken from the exposed or covered part of the body.

DISCUSSION

The presence of type I and type III collagens in the dermis was demonstrated biochemically by Epstein (14) and Chung et al. (15) and immunohistochemically by Nowack et al. (16). The immunohistochemical study indicated that antibodies against type I collagen reacted with all portions throughout the entire dermis and that antibodies against type III collagen reacted with the papillary dermis but not with the coarse bundles of the reticular dermis (1). Immunoelectron microscopic study by Fleischmajer et al. (2) supported this view. Epstein & Munderloh (3), however, biochemically determined the quantitative ratio of type I to type III collagen, and reported that this ratio was nearly constant regardless of the depth of the dermis. Recent biochemical analysis (4) have supported the data of Epstein & Munderloh (3). In our experiment, both type I and type III collagens were found to be diffusely distributed throughout the entire dermis. Before deciding the optimum dilution of this study, we had performed dilution experiments. Primary and secondary antibodies were applied at various dilution (1:1, 1:5, 1:10, 1:20, 1:40, 1:80, 1:200, 1:400, 1:1000). We have confirmed that the distribution patterns do not change at any dilution. We had also performed tissue denaturing experiments with guanidine chloride and urea and observed the same distribution patterns of type I and III collagens. These results are not in agreement with those of Meigel et al. (1). While the reasons for this

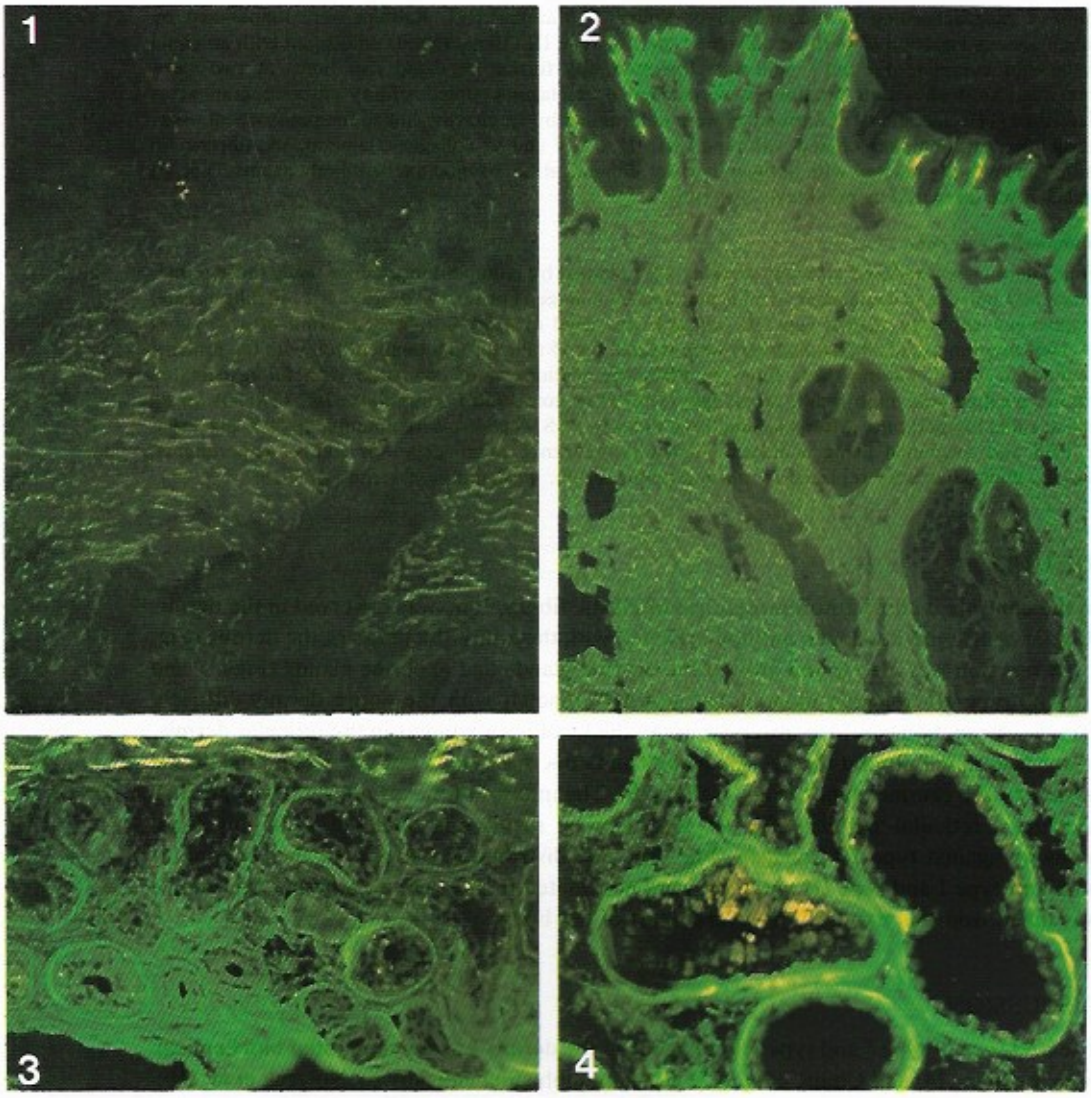
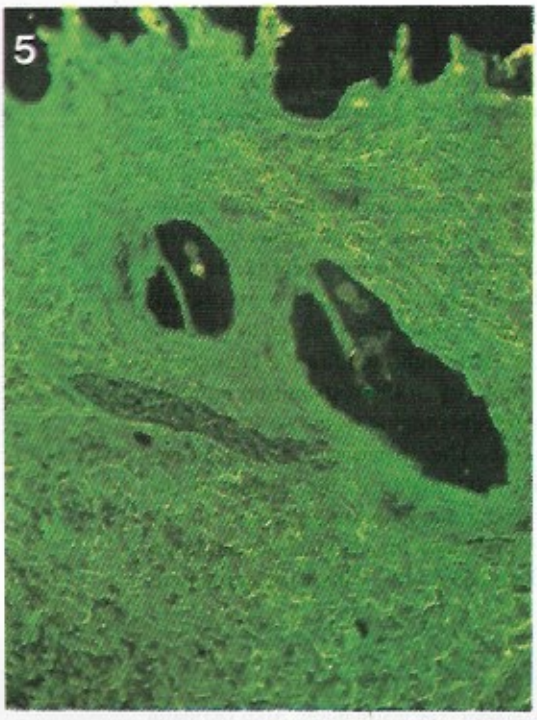


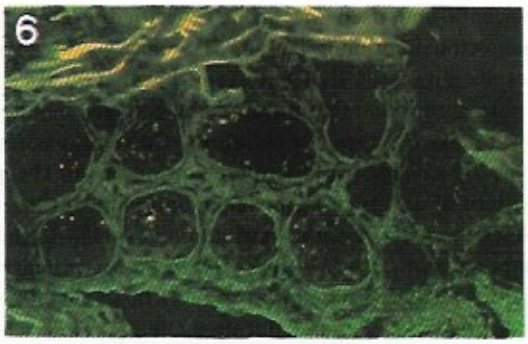
Fig. 1. Control preparation (normal rabbit serum). No immunostaining was observed except yellow autofluorescence of elastic fibers (×80).

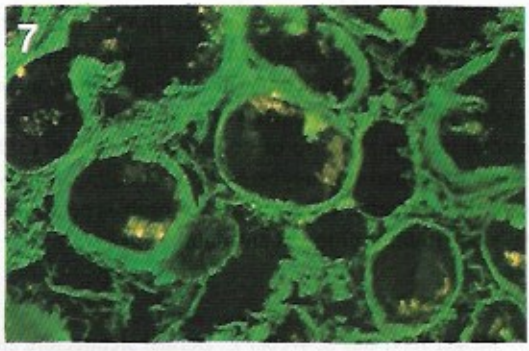
Fig. 2. Indirect immunofluorescence (IF) staining of normal human skin with antibodies to type I collagen (×80).

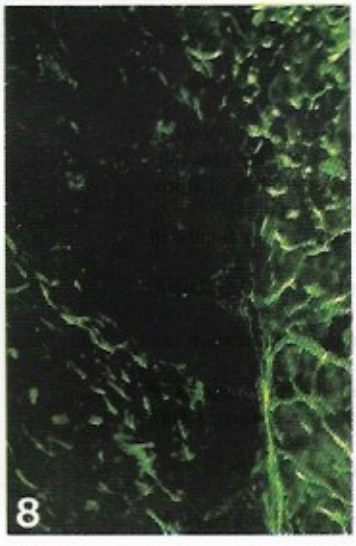
Figs. 3 and 4. Anti-type I collagen antibody reacted in circular pattern around the eccrine (Fig. 3) and apocrine glands (Fig. 4) (×160).

difference are not completely clear, they may be due to the quality of antibodies. Meigel et al. obtained their antibodies by immunizing rats with collagens isolated from calf skin. Although their study was based on the assumption that bovine and human collagens immunologically cross-react, the use of human collagen antibody would allow a more accurate evaluation. The immunofluorescence technique may yield inconsistent results if the antigens used for the production of antibodies differ in type, purity, or species. Therefore, we prepared the primary antibodies using collagens derived from the human









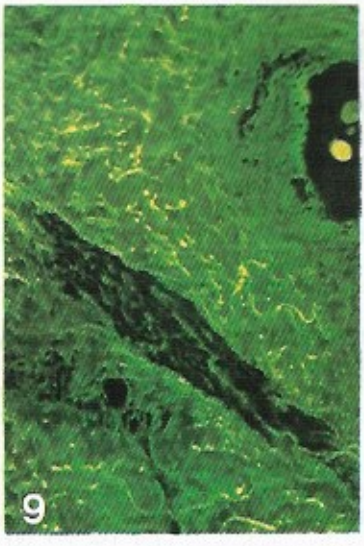


Fig. 5. Indirect IF staining of normal human skin with monoclonal antibodies to type III collagen. Reaction is observed diffusely in the whole dermis (×80).

Figs. 6, 7 and 8. Anti-type III collagen monoclonal antibody reacted in circular pattern around the eccrine (Fig. 6) and apocrine glands (Fig. 7) but not around the ductal portion of the eccrine gland (Fig. 8) (×160).

Fig. 9. Anti-type III collagen monoclonal antibody reacted in reticular pattern in the arrector pili (×95).

Fig. 10. Anti-type III collagen polyclonal antibody reacted with the whole dermis (×55).

placenta and used the affinity purified $F(ab')_2$ fraction as the secondary antibody. A monoclonal antibody to type III collagen was also prepared. Using these antibodies, our results suggested that both type I and type III collagens are distributed throughout the entire dermis and that thick coarse collagen bundles in reticular dermis are composed of at least these two types of collagens. Conventional immunohistological studies indicated that type III collagen is distributed primarily in the papillary dermis and that the reaction pattern of anti-type III collagen antibodies in the reticular dermis is linear or threadlike. Since the pattern of its distribution is similar to that of the reticular fibers shown by silver impregnation, it was considered to be the primary component of these fibers (2, 16, 17). However, our results do no support this view. Type I and type III collagens were present in nearly equal amounts during the fetal period, while type I collagen decreases to 15–20% neonatally, and type I collagen increases with age (14). Although quantitative changes in these collagens were not examined in this study, the similarity in the distribution patterns of type I and type III collagens in the skin samples of subjects ranging from two to 85 years of age indicates uniformity in their distribution patterns. The structural differences between type I and type III collagens and their spacial relationship in the dermis of the normal human skin are of considerable interest. Our preliminary immunoelectron microscopic examination suggested that all collagen fibrils which have the cross-banded pattern have the antigenic determinant of both type I and III collagen (18). This is in agreement with our immunofluorescent data.

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