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Monoclonal Antibodies to Collagens for Immunofluorescent Examination of Human Skin

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Monoclonal antibodies, specific for each of human types I, III, IV and V collagens, were produced and shown to be suitable for immunofluorescent studies of human skin. The antibodies showed that types I and III collagens were abundant and distributed throughout the dermis. The distribution of type III collagen appeared different in the papillary compared with the reticular dermis, although an increased concentration of type III collagen in the papillary dermis could not be unambiguously established. Type V collagen, which could be visualised only after acid-pretreatment of sections, was also distributed throughout the dermis, but appeared to be in higher concentrations around cells. Type IV collagen was observed specifically in the basement membrane associated with the dermal/epidermal junction. ***Key words: Immunohistology; Genetically distinct collagens; Specific identification.***

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The development of antibodies against collagens has provided a powerful method for examining the distributions of these connective tissue proteins in human skin (1). Although polyclonal antibodies against collagens have been described (2, 3), problems resulting from the lack of specificity of these antibodies and variations between different batches make such preparations of limited value. Collagens are extremely poor immunogens and so very low quantities of impurities can elicit the major antigenic response. These impurities could be serum proteins, or even other collagen types (4, 5, 6). Also, the collagen family is structurally very homologous, so cross-reactivity of antibodies between different collagen types can occur. While absorption of polyclonal antibodies onto collagen affinity columns can potentially improve the specificity of these antibodies (7), monoclonal antibodies to collagens provide the best way of obtaining specific antibodies which have reproducible properties and are not batch dependent. The extremely low immunogenicity of the collagens makes the production of monoclonal antibodies technically difficult. A monoclonal antibody suitable for examining type III collagen in human skin has recently been described (3). In addition, monoclonal antibodies to collagen types IV and V have been described which may be useful for similar work (2). The present study describes a panel of new monoclonal antibodies to these individual collagen types, and also to type I collagen, which are suitable for examining the distribution and organization of each collagen type in human skin using immunofluorescence.

MATERIALS AND METHODS

Monoclonal antibodies

Pepsin soluble collagens from human placenta were obtained from Sigma Chemical Co. (St. Louis, MO) and were further purified by fractional salt precipitation at acid and neutral pH (8, 9). Monoclonal antibodies to types I, III, IV and V collagens were prepared using SJL/J mice and NS1 myeloma cells as previously described (10, 11). Antibody specificity was established by screening with an enzyme linked immunoabsorption assay (ELISA) (12) and by electrophoresis and electroblotting of collagens using a non-denaturing polyacrylamide gel electrophoresis system (13). A panel of connective tissue proteins, collagen types I, III, IV, V, elastin, fibronectin (Sigma Chemical Co., St. Louis, MO), or laminin (Collaborative Research Inc., Lexington, MA), was used for screening. Monoclonal antibodies that were specific for each of human collagen types I, III, IV and V were selected. Full details of the preparation and characterisation of these antibodies will be presented elsewhere.

Immunofluorescence

Human skin samples from patients without any connective tissue disorder, in an age range 1–4 years, were obtained through the Orthopaedic Research Unit of The Royal Children's Hospital, Parkville, and had been taken with informed parental consent in accordance with the ethics procedures of the Hospital. Sections, 5 microns thick, were cut from frozen skin samples using a freezing microtome, and were examined using the panel of monoclonal antibodies, using undiluted culture supernatants. After incubation with antibody, sections were washed twice for 10 min in 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4 (PBS). They were then visualised with affinity purified, fluorescein isothiocyanate conjugated, sheep anti-mouse antibody (Silenus Laboratories, Melbourne) diluted 1/50 in PBS. After a further two washes for 10 min in PBS, sections were mounted in glycerol/water (9 : 1,

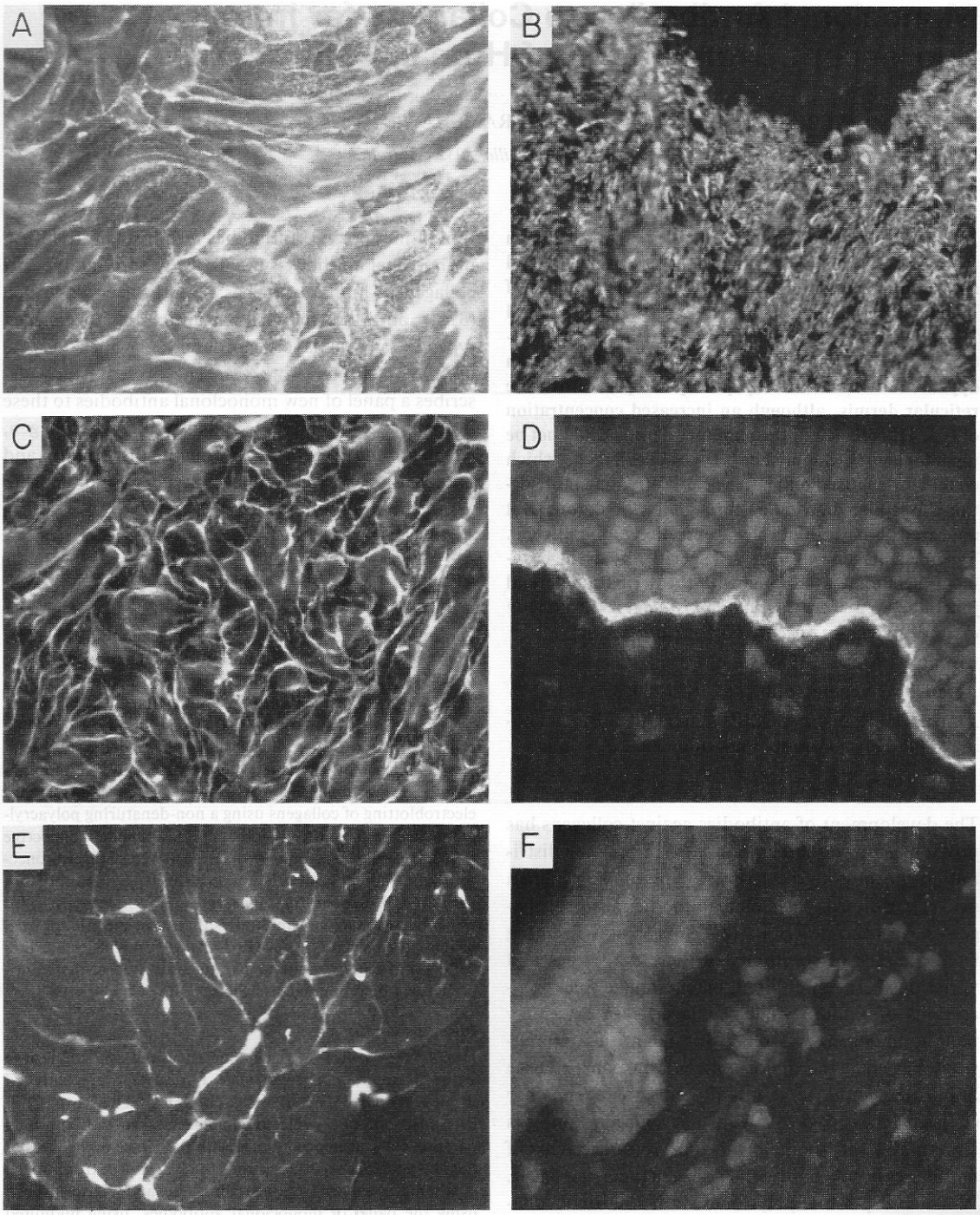

Fig. 1. Immunofluorescent examination of human skin sections from a single patient aged 22 months. (A) Reticular dermis examined with anti-type I collagen antibody (×40). (B) Papillary dermis examined with anti-type III collagen antibody (×80). (C) Reticular dermis examined with anti-type III collagen antibody (×40). (D) Dermal/epidermal junction examined with anti-type IV collagen antibody (×80). (E) Reticular dermis examined with anti-type V collagen antibody (×40). (F) Control of papillary dermis examined without addition of specific antibody (×40). Colour copies of this figure are available on request to the authors.

v/v) containing 1 mM 1,4-phenylenediamine. Sections were examined using a Leitz Ortholux II microscope with an L2 filter block. Fluorescence, after excitation at 450–490 nm, was visualised through a band pass filter, 525/20 nm. In control experiments spent culture supernatants from NS-1 cells or from an irrelevant hybridoma line were used or the specific monoclonal antibody was omitted.

RESULTS AND DISCUSSION

The specific monoclonal antibodies against types I, III, IV and V collagens were all suitable for immunofluorescent examination of human skin (Fig. 1), and, unlike polyclonal antibodies, can provide a limitless and reproducible source of antibodies for these studies. In all control experiments, only very low levels of fluorescence were observed, principally associated with cells. None of the antibodies gave any staining of the epidermal layer, although the anti-type IV antibody stained the epidermal/dermal junction.

Types I and III collagens represent the major collagen types present in skin, and are distributed throughout all zones. The immunofluorescent studies using the antibody to type I collagen showed reaction throughout the entire thickness of the dermis, with large fibre bundles being particularly evident in the reticular dermis (Fig. 1 A).

The type III collagen showed distinct differences in organisation between the papillary and reticular dermis. In the papillary dermis the collagen had a somewhat amorphous appearance (Fig. 1 B) while in the reticular dermis it had a fibrous appearance and was apparently associated with exterior of the larger fibre bundles of type I collagen (Fig. 1 C). Type III collagen with a fibrous appearance was also observed associated with specific structures in the skin, for example, hair follicles and vessels.

In previous immunofluorescence studies using polyclonal antibodies, type III collagen has been described as being in greater concentration in the papillary dermis (14). In the present study, type III collagen was seen throughout the dermis, but differential concentrations could not be unambiguously determined. Similar results were reported in another study where a monoclonal antibody to type III collagen was used (3). Chemical studies of the distribution of type III collagen in human skin indicated a uniform distribution (15), while those on bovine skin showed a higher concentration of type III collagen in the papillary dermis (16). The use of immunofluorescence to quantitate or compare relative concentrations of collagen types must be treated with a degree of caution. Variations in antibody dilution could lead to changes of staining intensity, although previous studies suggested that this may not lead to staining variations (3). Potentially much greater problems arise, however, from inefficiencies of staining due to inaccessibility of antibody binding sites, because of collagen packing or masking by other connective tissue components such as proteoglycans. Thus, for example, type V collagen can often only be visualised after pretreatment of sections with acid (17). This was found in the present study of human skin, using 0.1 M acetic acid for 15 min followed by washing in PBS. Type V collagen, which is present in skin in low concentrations (18), was observed throughout the dermis, but appeared to be mostly associated with cells (Fig. 1 E). The other collagen examined was type IV, which is also a minor component (18). This collagen, however, did not show a generalised distribution, but was specifically located at the dermal/epidermal junction, being associated with the basement membrane (Fig. 1 D) (19). These data show that a panel of highly specific monoclonal antibodies to individual collagen types has been generated and that these antibodies are suitable for immunohistological investigations of collagen organisation in human dermis.

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