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Localization of Vitronectin (S-protein of Complement) in Normal Human Skin

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Vitronectin, now known to be identical to serum spreading factor and to S-protein of complement, is a multifunctional glycoprotein involved in the adhesion and spreading of cells and in the complement and coagulation pathways. The distribution of vitronectin in normal human skin was investigated with immunofluorescence using polyclonal antibodies, and with an avidin-biotin peroxidase complex technique, using polyclonal as well as monoclonal antibodies. Vitronectin immunreactivity was found to be localized on the elastic fibres in the dermis. Both the thin fibres in the papillary dermis and the thicker elastic fibres in the reticular dermis were stained. No crossreactivity was found between vitronectin and serum amyloid P component, known to bind to elastic fibres. The two proteins were immunohistochemically localized to the same structures in the skin. The distribution of vitronectin in the dermal tissue established in this study provides a basis for further studies of the function and behaviour of vitronectin in health and disease. (Received June 5, 1986.)

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Vitronectin, also known as serum spreading factor, is one of several proteins capable of promoting adhesion of cells to extracellular substrata or to other cells (1, 2, 3). The best characterized of the adhesive proteins is fibronectin, others being chondronectin, laminin, collagen and the so-called cell adhesion molecules (CAMs) (4, 5).

Recently, the complete nucleotide sequence was determined of a cDNA clone for human vitronectin (6). Shortly afterwards, when the sequence of a cDNA clone coding for the human regulatory complement protein, S-protein, was elucidated, the two proteins were found to be identical (7). S-protein was originally described as a subcomponent of the soluble SC5b-9 complex of complement (8). It was later shown that it functions as an inhibitor of the cytolytic membrane attack complex of the complement system (9). In addition to this effect, it has been shown to interact with thrombin and antithrombin III (AT III) during coagulation, and may have a physiological role in the coagulation pathway since it abolishes the heparin stimulation of AT III inactivation of thrombin (10). The heparin binding site of vitronectin has been localized to a specific region, which is rich in basic amino acids (11). Since the identity between vitronectin, serum spreading factor and S-protein has been unequivocally demonstrated, for simplicity, the protein will be referred to as vitronectin throughout this paper.

Vitronectin is found in high concentrations in human plasma (12, 13). The protein has also been isolated from human fetal membranes, indicating the existence of a tissue form (14). With immunofluorescence techniques, it has been identified on the surface of thrombocytes and fibroblasts (14, 15), as well as in various human tissues (muscle, kidney, embryonal lung and skin) (14).

A systematic study on the exact localization of vitronectin in human tissue has yet to be done. In this study we have investigated the localization of vitronectin in normal human

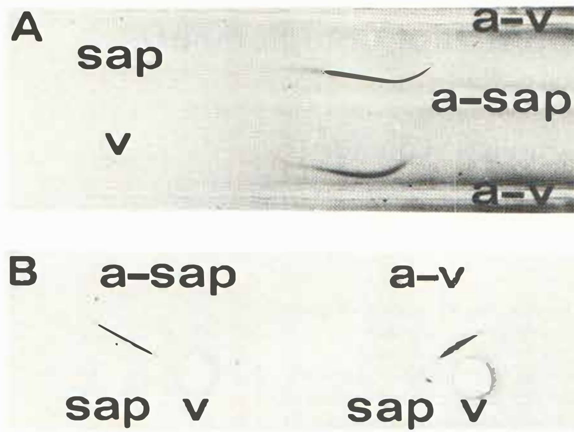

Fig. 1. Immunelectrophoresis (*A*) and double immunodiffusion (*B*) with purified vitronectin (*v*), purified SAP (*sap*), anti-vitronectin (*a-v*), and with anti-SAP (*a-sap*).

skin, correlating it with that of serum amyloid P-component (SAP), a plasma protein known to bind to the elastic fibres in the dermis (16, 17).

MATERIALS AND METHODS

Biological tissue and fixation procedure

Skin biopsies from ten adult individuals were studied. Two different ways of handling the specimens after surgery were used: they were either instantly frozen or were immersed in a transport medium (55% (w/v) ammonium sulphate, 5% of 0.1 M ethylmaleimid, 2.5% of 1 M potassium citrate and 5% of 0.1 1 M magnesium sulphate) being washed within 48 h in transport medium lacking ammonium sulphate and then immediately frozen in chlordifluormethane R22 at the temperature of liquid nitrogen. The two ways of handling the specimens gave the same final results. All specimens were stored at −70°C until processed. Cryostat sections, between 4 and 10 μm thick, were cut and fixed in acetone for 20 min at 4°C, consecutive sections being treated to localize elastin, SAP or vitronectin in turn.

Proteins and primary antisera

Vitronectin was isolated from human plasma, using the method developed for S-protein described by Dahlbäck & Podack (12). The sample used in the adsorption experiment was one of the pools (pool Ia) characterized in detail in the original paper. Using another pool (pool IIa) a polyclonal antiserum was produced in rabbits. When tested against human plasma with immune electrophoresis (18), and crossed immune electrophoresis (19), the antiserum was shown to be contaminated with antibodies against prothrombin. When these antibodies had subsequently been removed by adsorption in a prothrombin-sepharose column, the antiserum only demonstrated a single precipitin line on being tested against human plasma with immune electrophoresis and with crossed immune electrophoresis. Moreover, on Western blotting (20), it only reacted with vitronectin in plasma (1–50 μl plasma applied to the first dimension SDS-polyacrylamide slab gel electrophoresis). Unless otherwise stated, the working dilution used in the immunohistochemical experiments was 1 : 20000.

A mouse monoclonal antibody against vitronectin (called S-protein at Cytotech) was a kind gift of Dr Tamerius at Cytotech, San Diego, USA. The working dilution was 1 : 500 (2 μg/ml).

SAP was isolated as a by-product during the purification of C4b-binding protein from human plasma (21). It was eluted from the heparin-sepharose column with EDTA (see Fig. 1 in the original paper). The preparation was more than 95% pure as judged by agarose- and SDS/polyacrylamide-gel electrophoresis. A polyclonal antiserum against human SAP was obtained from Dakopatts a/s Copenhagen, Denmark, its working dilution in the immunohistochemical experiments being 1 : 20000 unless otherwise stated.

Antiserum control procedures

The antisera against vitronectin and SAP had high titres and they still gave positive immunofluorescence, even when used at dilutions as high as 1 : 90000 or 1 : 270000. The specificities of the

polyclonal vitronectin and SAP antisera were tested against plasma proteins, both with immune-precipitation techniques and with Western blotting as discussed above. No cross-reactivity between the two antisera could be demonstrated, either with double immunodiffusion (Ouchterlony) analysis (22) or with immune electrophoresis using purified proteins (Fig. 1). Moreover, the specificities of the antisera-tissue subcomponent reactions were checked by adsorption experiments. Aliquots (100 µl) of the three antisera, diluted in PBS containing 2.5% BSA to their respective working dilutions, were incubated overnight with increasing amounts (0.01–100 µg) of purified vitronectin or purified SAP. In adsorption experiments no cross-reactivity was found between the anti-vitronectin and the anti-SAP antisera; the anti-vitronectin staining whether obtained with polyclonal or monoclonal antibodies remained unaffected even by the highest amount of SAP tested; similarly the anti-SAP staining was unaffected by vitronectin. The anti-vitronectin staining obtained both with the monoclonal anti-vitronectin antibody and the polyclonal antiserum was partly, strongly and completely inhibited by 0.03 µg, 0.3 µg and 3 µg purified vitronectin, respectively; the anti-SAP staining was partly, strongly, and completely inhibited by 0.01 µg, 0.1 µg and 1 µg purified SAP, respectively.

Immunohistochemical techniques

Immunofluorescence. Cryostat sections were fixed with acetone for 20 min at 4°C and were then incubated with antiserum for 30 min, washed, and incubated with fluorescein-conjugated (FITC) goat antirabbit IgG for 30 min. The slides were examined under a Leitz fluorescence microscope with transillumination. Photographs were taken with a recently developed scanning method (23).

Immunoperoxidase staining. The avidin-biotin peroxidase complex technique was used as described by Hsu et al. (24).

Elastin staining

Elastic fibres were stained over night with orcein (25). As an additional test of the identity of the fibres stained with the polyclonal anti-vitronectin antiserum in the immunofluorescence technique, after having been photographed in the fluorescence microscope, the slides were formalin fixed and then elastin stained.

RESULTS

Indirect immunofluorescence staining of normal dermal tissue using a polyclonal vitronectin antiserum, resulted in an immunoreactivity associated with dermal fibrillar structures (Fig. 2). The staining pattern was quite distinct, and its distribution suggested that the immunoreactive material was associated with the elastic fibres. This was confirmed by the identical staining pattern obtained when orcein staining was performed on the same tissue section (Fig. 2). Similar staining patterns were obtained with the avidin-biotin peroxidase complex using both the polyclonal antiserum and a monoclonal antibody (Fig. 3*c* and *d*).

Since it had recently been shown that another plasma protein, SAP, is associated with the elastin microfibrils (16, 17), we compared, in consecutive cryostat sections, the anti-Vitronectin staining with that of anti-SAP and with a standard elastin staining. Similar staining patterns were obtained with all these alternatives, suggesting that Vitronectin is associated with the same dermal structures as SAP (Fig. 3*a*, *b*, *c* and *d*).

The thin fibres in the papillary dermis, orthogonal to the dermo-epidermal junction, appeared to be stained homogeneously, whereas the thicker elastic fibres in the reticular dermis were seen as tubelike structures in which the staining was confined to the periphery (Fig. 3*e* and *f*). No specific staining was observed of the epidermis, nor of the dermo-epidermal basement zone.

DISCUSSION

Hayman et al. (14), using a monoclonal antibody against vitronectin, have reported it to be present in dermal tissue as well as in tissues from several other organs. Using an immunofluorescence technique, their monoclonal antibody was found to associate with 'loose connective tissue' in dermis, but no further details of its localization were reported.

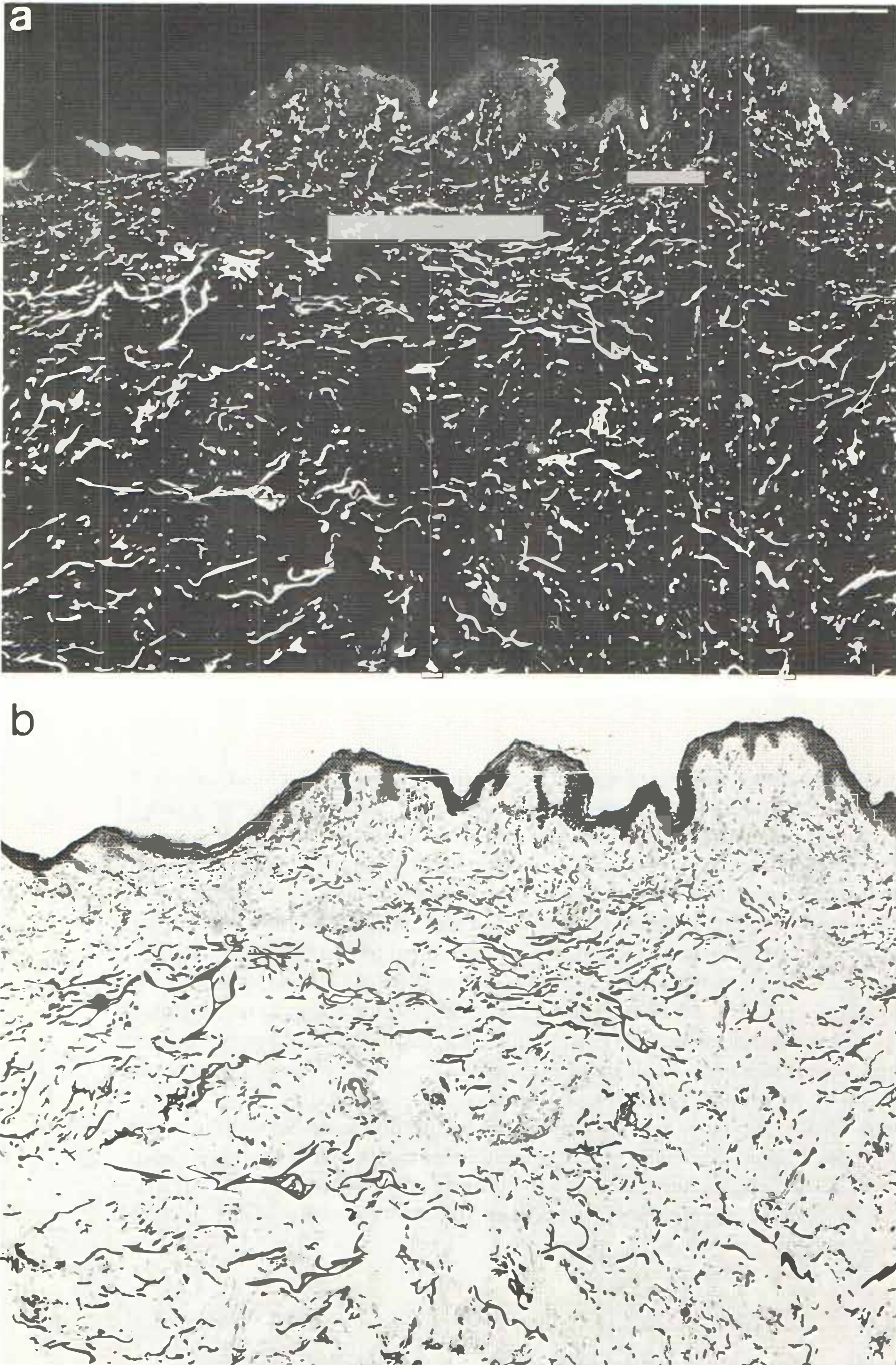

Fig. 2. Immunofluorescence staining of normal human skin with polyclonal anti-vitronectin antiserum (working dilution 1 : 3 000) (*a*) and orcein staining of the same tissue section (*b*). Bar = 0.2 mm.

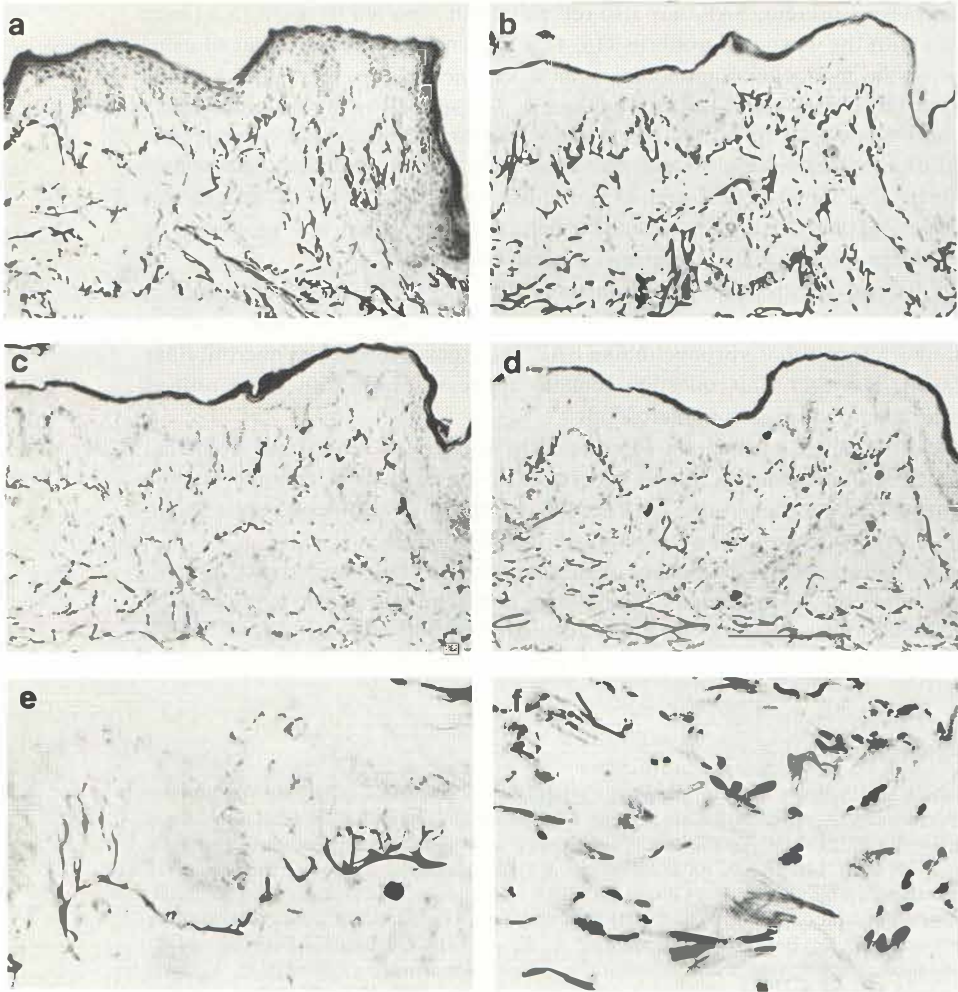

Fig. 3. Consecutive sections of normal human skin stained with orcein (*a*), and with the avidin-biotin immunoperoxidase technique using either anti-SAP (working dilution 1 : 10000) (*b*), polyclonal anti-vitronectin (*c*), or monoclonal anti-vitronectin (*d*). Higher magnification of subepidermal thin fibres stained with polyclonal anti-vitronectin (*e*) and of thicker, tubelike, elastic fibres in reticular dermis stained with monoclonal anti-vitronectin (*f*). *a, b, c, d*×350. *e, f*×875.

Vitronectin has been demonstrated to play an important role in vitro in cell adhesion and cell spreading (1–3). These functions are also associated with fibronectin, another plasma protein occurring in a tissue form (4).

The recent discovery that S-protein of complement and vitronectin are one and the same has shown vitronectin to be a multifunctional protein which, in addition to its adhesive properties, is capable of interacting in the complement and the coagulation pathways (7). This new concept indicates that vitronectin's inhibitory effect on the membrane attack complex of complement and its effect on thrombin may be localized to certain cells and/or certain matrix constituents. In view of the importance of establishing the distribution of vitronectin in tissue, the present study was designed to ascertain its distribution in skin.

We are now able to report that in human dermis vitronectin is associated with the elastic

fibres. Another plasma protein, SAP, has also recently been reported to occur in a tissue form associated with the elastic microfibrils (16, 17). The immunostaining obtained using the polyclonal or the monoclonal antibodies against vitronectin was very similar to the immunostaining obtained with the anti-SAP antiserum. The specificity of the immunoreactivity of vitronectin and SAP in dermal tissue was corroborated by control experiments. Using several different techniques, the polyclonal antisera against vitronectin and against SAP were shown to be monospecific and, of particular importance, no cross-reactivities between the two antisera or proteins could be detected. Nor could any reactivity be detected between the monoclonal anti-vitronectin antibodies and SAP.

The staining pattern of vitronectin found in this study, i.e., apparently homogeneous staining of the thin papillary oxytalan fibres, but maximum staining of the periphery of the thicker fibres, may suggest that vitronectin like SAP, is associated with the microfibrillar portion of elastin. However, this question remains to be resolved with more refined techniques such as immuno-electron microscopy.

The tissue distribution of vitronectin reported here appears to be distinct from that previously reported for fibronectin which was found to be associated mainly with the epidermal-dermal basement membrane, with dermal collagen and with vessel walls and to be distributed in a reticular pattern in the papillary dermis (26, 27).

The presence of vitronectin on the surface of elastic fibres may be of importance for cell-tissue interaction and for cell migration in the human skin. The functional significance of the tissue-associated form of vitronectin in the regulation of fibrin deposition and inhibition of the complement membrane attack complex also remains to be elucidated.

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