Immunohistochemical Studies on Vitronectin in Elastic Tissue Disorders, Cutaneous Amyloidosis, Lichen ruber planus and Porphyria

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Vitronectin, identical with serum-spreading factor and S-protein of complement, is a glycoprotein present in both plasma and tissue. It stimulates cell adhesion and spreading and affects the complement and coagulation pathways. Vitronectin immunoreactivity was recently found in conjunction with dermal and renal elastic fibres, in renal amyloid deposits in cases of AL- and AA-amyloidosis, and in sclerotic glomerular lesions. Skin specimens from lesions of patients with selected skin diseases were investigated with an avidin–biotin peroxidase technique using both monoclonal and polyclonal anti-vitronectin antibodies and an alkaline phosphatase anti-alkaline phosphatase technique using monoclonal anti-vitronectin antibodies. Vitronectin immunoreactivity was found in association with the abnormal elastic tissue in solar elastosis and pseudoxanthoma elasticum. It was also found in conjunction with dermal amyloid deposits in primary localized cutaneous amyloidosis and in Civatte bodies in cases of lichen ruber planus. In cases of erythropoietic protoporphyria and porphyria cutanea tarda, hyaline perivascular deposits also demonstrated positive vitronectin immunoreactivity. The presence of vitronectin immunoreactivity not only in normal and degenerated elastic fibres but also in various pathological tissue deposits suggests that vitronectin occurs both in elastic fibres and in different types of abnormal protein deposits. *Key words: Solar elastosis, pseudoxanthoma elasticum.* (Received September 21, 1987.)

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Vitronectin, also known as serum-spreading factor and S-protein of complement, is a multifunctional plasma and tissue glycoprotein. It has adhesive properties and promotes the attachment and spreading of cultured fibroblastic cells onto tissue culture dishes in vitro (1). It also acts as an inhibitor of the cytolytic membrane attack complex of the complement system (2) and may have a regulatory effect in the coagulation pathway, since it inhibits the inactivation of thrombin by antithrombin III (3).

Using immunohistochemical techniques, we have demonstrated the presence of vitronectin in conjunction with the elastic fibres of human dermis (4). Recently, we also found vitronectin immunoreactivity in association with the elastic tissue in renal arteries, with renal amyloid deposits in AA- and AL-type amyloidosis in the kidney and with sclerotic lesions of benign nephrosclerosis (5).

The distribution of vitronectin immunoreactivity in the dermal elastic fibre network corresponded to that found for serum amyloid P component (SAP), fibrillin and NKH-1 (6–8), all of which have been demonstrated to bind to the elastin-associated microfibrils rather than to the amorphous part of the elastic fibre.

The purpose of the present study was to determine the distribution of vitronectin immunoreactivity in various skin disorders. Skin specimens from patients with solar

elastosis, pseudoxanthoma elasticum (PXE), primary localized cutaneous amyloidosis (PLCA), lichen ruber planus and with erythropoietic protoporphyria (EPP) and porphyria cutanea tarda (PCT) were investigated.

MATERIALS AND METHODS

Biological tissue

Specimens were obtained from skin lesions of patients with cutis rhomboidalis nuchae (7 cases), actinic keratosis (5 cases), PXE (3 cases), papular type of PLCA (2 cases), macular type of PLCA (1 case), lichen ruber planus (5 cases) and from the dorsum of the hands in cases of EPP (5 cases) and of PCT (2 cases). In the cases of solar elastosis and porphyria, the patients were over 40 years of age. The different diagnoses had been established on both clinical and histological grounds and in the cases of porphyria also on the basis of biochemical criteria. After hematoxylin-eosin staining, specimens were subjected to routine histological examination. Special stainings such as standard elastin staining using orcein, von Kossa's staining, congo red staining, and periodic acid Schiff (PAS) staining were used when necessary.

In the specimens with solar elastosis, characteristic changes of elastosis with thick irregular, curled fibres or amorphous material in the upper dermis with lack of oxytalan fibres were seen after staining with orcein. The specimens with PXE had pathological granular material in the lower part of the dermis that was stainable with both orcein and von Kossa's stain. In specimens from the 3 patients with PLCA, congophilic amorphous material was found in the papillary dermis, that showed green birefringence when examined in polarized light. In the cases of lichen ruber planus, colloid bodies, so-called Civatte bodies, were found in the area of the dermal–epidermal junction, and there was a band-like cellular infiltrate of lymphocytes beneath this area. The specimens from the patients with EPP and PCT all had deposits of hyaline, PAS-positive material in association with vessel walls in the upper dermis. The surrounding dermal tissue showed elastotic changes.

Fixation procedure

The specimens were divided into two parts, one of which was formalin-fixed, the other immersed in a transport medium (550 g ammoniumsulfate added to 1 litre 25 mM potassium citrate, 5 mM *N*-ethylmaleimide, 5 mM magnesium sulfate), being washed within 48 h in transport medium lacking ammonium sulfate and then immediately frozen in chlordifluormethane R22 at the temperature of liquid nitrogen. The frozen specimens were stored at −70°C, and were acetone-fixed before being processed.

From one of the cases of papular PLCA and from one of the cases of PXE, only formalin-fixed specimens were obtained. Only frozen specimens were obtained from the patients with lichen ruber planus, EPP and PCT.

Proteins and primary antisera

Vitronectin was isolated from human plasma with the method developed for S-protein described by Dahlbäck & Podack (9). The polyclonal antiserum against vitronectin has been characterized previously (4). Monoclonal antibodies against vitronectin was kindly donated by Dr Tamerius at Cytotech, San Diego, USA. A polyclonal antiserum against serum amyloid P component (SAP) was obtained from Dakopatts a/s Copenhagen. The working dilution of all antibodies was 1:10000. Sequence-specific antibodies against amyloid A, used as a marker of the amyloid A type of amyloidosis, was the kind gift of Dr A. Grubb, Department of Clinical Chemistry, Malmö General Hospital, Malmö. The working dilution was 1:50.

Antiserum control procedures

Anti-vitronectin and anti-SAP antisera were tested for specificity as described earlier with immunoprecipitation and Western blotting techniques and with crossed adsorption experiments (4).

To further corroborate the specificities of the antisera–tissue subcomponent reactions, blocking experiments were done using monoclonal anti-vitronectin preadsorbed with purified vitronectin on specimens from patients with solar elastosis, PXE, PLCA, lichen ruber planus, EPP and PCT.

Immunohistochemical techniques

The avidin–biotin peroxidase complex technique and the alkaline phosphatase anti-alkaline phosphatase (APAAP) complex technique was used as reported earlier (5). Specimens from the patients with lichen ruber planus were also analysed with immunofluorescence using fluorescein-conjugated (FITC) rabbit antihuman IgM. Some of the specimens from cases of elastosis and PXE were studied with the

following sequential staining procedure: in the first reaction the monoclonal anti-vitronectin antibody was used in the APAAP complex technique, which produced a blue colour on the immunoreactive structures. After photography, the slides were left in 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5, for 12 h at 37° to detach the coverslips. Then the slides were treated with xylol for 3 min at room temperature, a procedure which removed the blue colour; in the second reaction the slides were stained with standard elastin staining procedure using orcein. This procedure made it possible to compare the anti-vitronectin staining with orcein staining.

RESULTS

Elastosis

Skin specimens were obtained from the neck of 7 patients with cutis rhomboidalis nuchae, and from the lesions of 5 patients with actinic keratosis. Elastotic material in the upper part of dermis was immunostained with anti-vitronectin in all the specimens, using monoclonal and polyclonal anti-vitronectin antibodies in the avidin–biotin peroxidase complex technique and monoclonal anti-vitronectin antibodies in the APAAP technique. Immunostaining of the elastotic material was as strong when formalin-fixed specimens were used as when acetone-fixed specimens were used. This was in contrast to the results with normal elastic fibres, which were weakly or not at all stained with anti-vitronectin when formalin-fixed specimens were used. Similar immunostaining of the elastotic material was obtained using anti-SAP antibodies in the avidin–biotin peroxidase complex technique. Using a sequential staining technique, it was demonstrated that the same structures were stained both by the monoclonal anti-vitronectin antibodies in the APAAP technique and in a standard elastin staining procedure (Fig. 1 *a, b*).

Pseudoxanthoma elasticum

Skin specimens were obtained from 3 patients with PXE, in all of which abnormal granular material, stainable with both orcein and von Kossa's stain, was found in middle and lower part of the dermis. This material was immunostained with both polyclonal and monoclonal anti-vitronectin antibodies in the avidin–biotin peroxidase complex technique and with monoclonal anti-vitronectin antibodies in the APAAP technique.

Immunostaining of the granular material was as strong when formalin-fixed specimens were used as when acetone-fixed specimens were used. Normal elastic fibres, including oxytalan fibres, in the upper dermis were immunostained with both monoclonal and polyclonal anti-vitronectin when frozen specimens were studied, but faintly or not at all stained, when formalin-fixed specimens were used. Using a sequential technique, the granular material was demonstrated to be stained both with monoclonal anti-vitronectin using the APAAP technique and with a standard elastin staining procedure (Fig. 2 *a, b*). Since the specimen used for photographic purposes was formalin-fixed, no oxytalan fibres were seen. Positive immunoreactivity of the granular material was also obtained with anti-SAP antiserum using the avidin–biotin peroxidase complex technique (Fig. 3).

Primary localized cutaneous amyloidosis

Since vitronectin immunoreactivity has been found in association with systemic amyloidosis, its distribution in skin specimens from lesions of 3 patients with PLCA was investigated. The specimens demonstrated congophilic amorphous deposits in the papillary dermis. This amorphous material could be labelled with anti-vitronectin, but not with an anti-AA antiserum, using the avidin–biotin peroxidase complex technique (Fig. 4 *a, b, c*). Polyclonal and monoclonal anti-vitronectin antibodies gave the same results in all 3 cases. The anti-vitronectin immunostaining of amyloid deposits was for unknown reasons stronger in the formalin-fixed specimens than in the acetone-fixed specimens. Similar immunostaining patterns were obtained with anti-SAP antiserum.

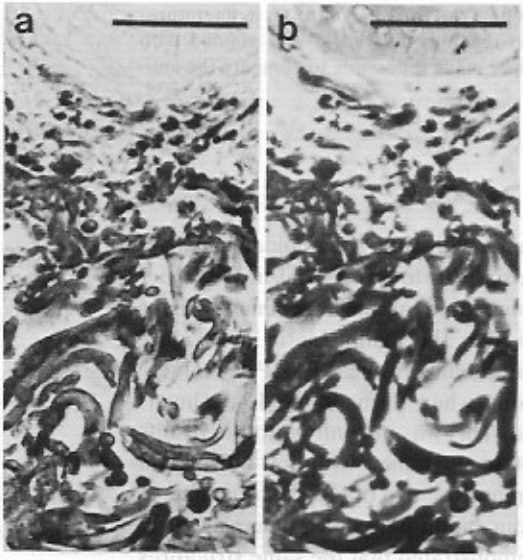

Fig. 1

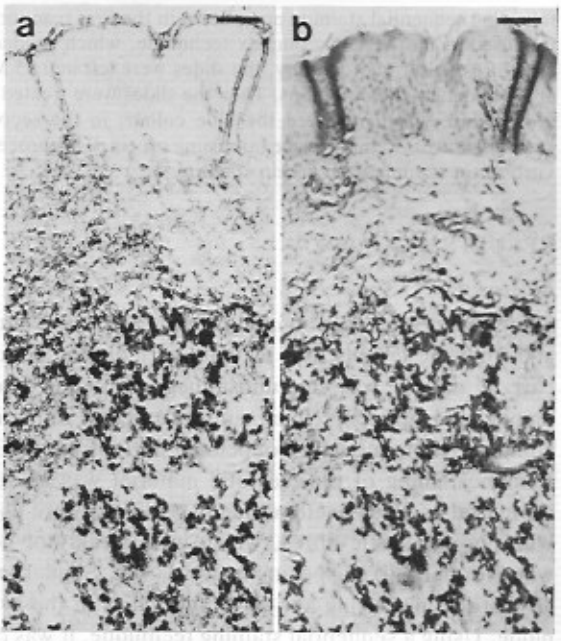

Fig. 2

Fig. 1. Vitronectin immunoreactivity in solar elastosis. Sequential staining of a section of skin with clinical and histopathological signs of solar elastosis with (*a*) monoclonal anti-vitronectin using the APAAP technique, and with (*b*) standard elastin staining procedure (orcein). Thick irregular curled fibres in the dermis are stained by both methods (bar=50 μm).

Fig. 2. Vitronectin immunoreactivity in PXE. Sequential staining of a section of skin with clinical and histopathological signs of PXE stained with (*a*) monoclonal anti-vitronectin using the APAAP technique, and with (*b*) standard elastin staining procedure (orcein). The granular pathologic material in the reticular dermis was stained with both techniques (bar=50 μm).

Lichen ruber planus

Owing to the reported similarities between deposits of cutaneous amyloid and Civatte bodies (10–15), skin specimens from patients with lichen ruber planus were included in the study. Civatte bodies were found in the dermal–epidermal junction area, and there was a band-like cellular infiltrate composed of lymphocytes and some histiocytes under this area in the specimens. The Civatte bodies demonstrated IgM reactivity when investigated with immunofluorescence. They had distinct vitronectin immunoreactivity, when both mono-

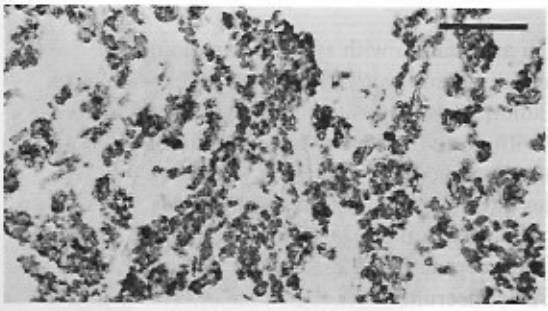

Fig. 3. SAP immunoreactivity in PXE. A section of a PXE skin lesion labelled with anti-SAP antiserum using an avidin–biotin peroxidase complex technique. Distinct immunoreactivity of granular material is to be seen in the reticular dermis (bar=50 μm).

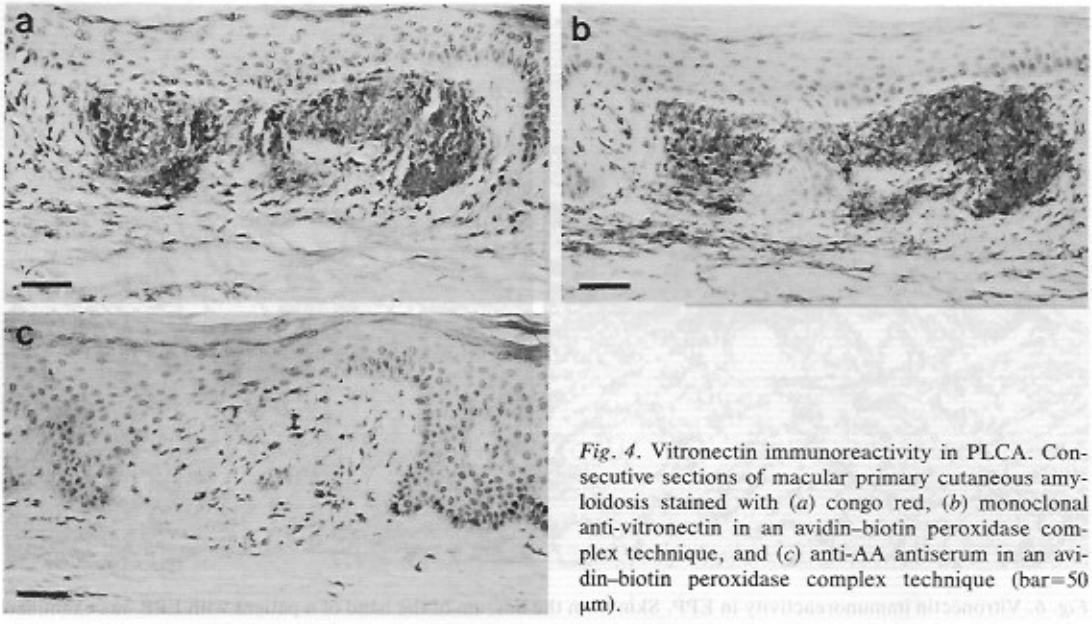

Fig. 4. Vitronectin immunoreactivity in PLCA. Consecutive sections of macular primary cutaneous amyloidosis stained with (*a*) congo red, (*b*) monoclonal anti-vitronectin in an avidin–biotin peroxidase complex technique, and (*c*) anti-AA antiserum in an avidin–biotin peroxidase complex technique (bar=50 μm).

clonal and polyclonal antibodies were used (Fig. 5) and could also be immunostained with anti-SAP. There was neither anti-vitronectin nor anti-SAP labelling of the dermal inflammatory cell infiltrate.

Erythropoietic protoporphyria and Porphyria cutanea tarda

Since SAP and fibronectin immunoreactivity has been reported in PAS-positive deposits in cutaneous vessel walls in cases of EPP and of PCT, skin specimens from patients with these diseases were investigated (16). The thickened vessel walls in the upper part of dermis present in all cases of EPP (Fig. 6) and PCT (Fig. 7) showed positive vitronectin immunoreactivity using either monoclonal or polyclonal anti-vitronectin antibodies. In addition, there was strong immunostaining of elastotic material in the upper dermis and of normal elastic fibres in the lower part of dermis. The staining pattern was quite similar to that obtained using anti-SAP antiserum.

DISCUSSION

The pattern of vitronectin immunoreactivity in solar elastosis and PXE corresponded to that of standard elastic staining, suggesting that vitronectin is associated not only with

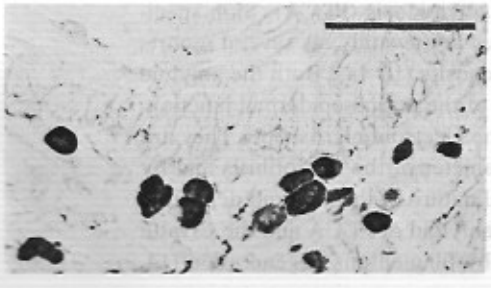

Fig. 5. Vitronectin immunoreactivity in lichen ruber planus. Staining of a lichen ruber planus skin lesion with monoclonal anti-vitronectin using the avidin–biotin peroxidase complex technique. Civatte bodies are distinctly immunostained (bar=50 μm).

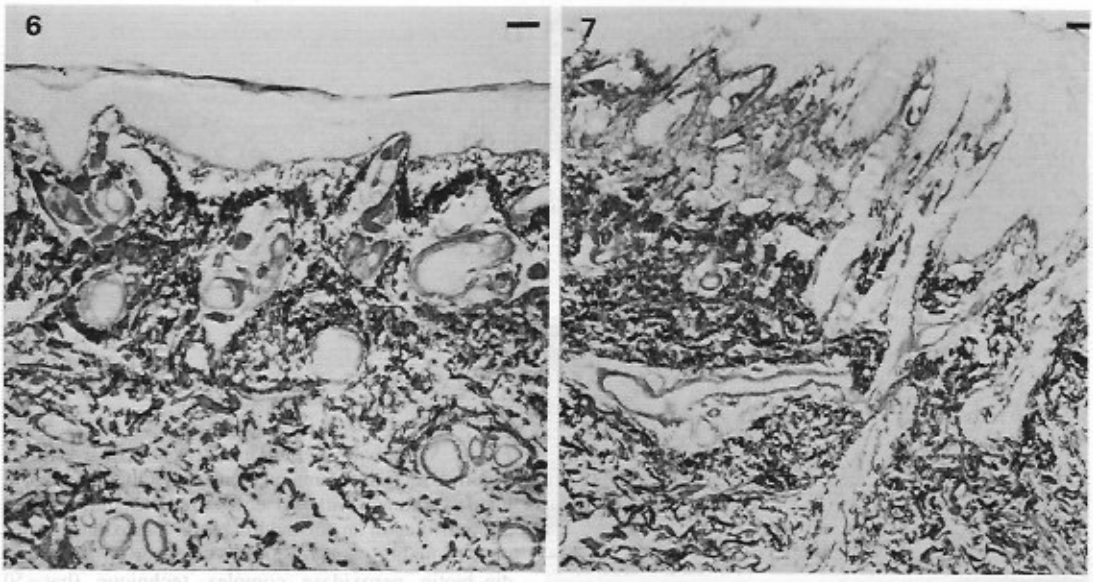

Fig. 6. Vitronectin immunoreactivity in EPP. Skin from the dorsum of the hand of a patient with EPP was examined with monoclonal anti-vitronectin using the avidin–biotin peroxidase complex technique. Thick perivascular deposits and surrounding elastotic tissue are stained (bar=50 μm).

Fig. 7. Vitronectin immunoreactivity in PCT. Immunostaining of skin section from the dorsum of the hand of a patient with PCT using monoclonal anti-vitronectin in an avidin–biotin peroxidase complex technique. Perivascular deposits and surrounding elastotic tissue are stained (bar=50 μm).

normal, but also with degenerated elastic tissue (4). The localization of the vitronectin immunoreactivity on the oxytalan fibres and on the periphery of larger elastic fibres in normal skin, indicates that, like SAP, fibrillin and NKH-1, vitronectin occurs in conjunction with the elastin-associated microfibrils. Immunoelectronmicroscopy studies are needed to clarify this. Ultrastructural studies of PXE and elastosis have demonstrated major changes in elastic fibres (17–20). In severely degenerated elastic fibres in PXE, paucity of microfibrils has been stated. However, proteins associated with the microfibrils still may be part of the degenerated material.

The exact chemical composition of the elastin-associated microfibrils is still not known, nor is their function (21). A 31 kDa glycoprotein, called MAGP, has been isolated and identified as the major antigen of these microfibrils. In addition, another elastic fibre antigen with MW 200 000 was described (22).

Since vitronectin immunoreactivity has been found in conjunction with renal amyloid deposits in the AA- and AL type of amyloidosis (5), we studied its distribution in skin specimens from lesions of primary cutaneous localized amyloidosis (PLCA). Skin specimens from patients with lichen ruber planus were included in the study, as several reports have described similarities between these two types of deposits (10–14). Both the amyloid deposits and the Civatte bodies are located in the region of the dermo-epidermal junction, and they are both fibrillar in structure when studied in the electron microscope. They are distinguishable from each other by the differences in diameter of the microfibrils and by differences in their arrangement (10). The exact chemical nature of the amyloid deposit in PLCA is not known. There are indications that both the amyloid of PLCA and the Civatte bodies of lichen planus are derived from keratinocytes by filamentous degeneration (14,

15). Immunoreactivity of immunoglobulins, mainly IgM, has been demonstrated in both types of deposits (12, 13).

The findings presented here indicate that vitronectin is associated both with the amyloid deposits of PLCA and with Civatte bodies. In addition, the results indicate association of vitronectin with the hyaline deposits in dermal vessel walls in cases of EPP and PCT.

Skin specimens from sun-exposed skin of patients with EPP and PCT were included in the study because of reports of SAP- and fibronectin-immunoreactivity in the PAS-positive deposits in cutaneous vessel walls in these diseases (16). In addition to reduplicated basal lamina, a finely fibrillar material is present in the abnormal deposits in cutaneous vessel walls in the sun-exposed skin in porphyria (23, 24). We were able to verify earlier reports of SAP immunoreactivity in conjunction with elastosis, amyloid deposits of PLCA and with hyaline deposits of cutaneous vessel walls in EPP and PCT (25, 26, 16). Our findings of positive SAP immunoreactivity in the abnormal dermal granular material of PXE, and in Civatte bodies of lichen ruber planus, differ for unknown reasons from those of earlier studies, where granular material in the lower dermis of one case of PXE, and colloid bodies in 5 cases of lichen planus were found to have faint or no SAP immunoreactivity (25, 26).

The recently isolated monoclonal antibody, NKH-1, developed using human subepidermal basement membrane zone as immunogen, was found to label the dermal elastic fibre network including the oxytalan fibres (8). Unlike anti-vitronectin and anti-SAP, it also stained the sub-basal lamina region continuously. It was shown to label amyloid deposits and elastotic material, as does anti-vitronectin, but not to stain the abnormal granular material in the lower dermis in cases of PXE.

Fibrillin, a 350-kDa glycoprotein, isolated from human fibroblast cell cultures was recently demonstrated immunohistochemically in conjunction with the elastin-associated microfibrils (7). The functions of SAP, NKH-1 and fibrillin remain unknown. Although vitronectin is known to possess potentially important functions in both the complement and coagulation pathways and in cell–tissue interactions, the physiological significance of the tissue form of vitronectin is unknown. Elastic fibres, amyloidosis, Civatte bodies, and hyaline deposits in vessel walls in cases of porphyria, all have a fibrillar component in common, suggesting that vitronectin may bind to microfibrils of different kinds. Ultrastructural studies are needed to elucidate this.

In view of vitronectin's ability to act as an inhibitor of the terminal cytolytic MAC complex in the complement system, it might be speculated whether binding of vitronectin to several different kinds of pathologic tissue deposits is an event secondary to activation of the complement system. There have been some reports supporting the hypothesis of complement activation being involved in the pathogenesis of porphyria (27–29); otherwise there are few indications of complement activation being involved in the pathogenesis of the examined diseases. Vitronectin has a documented tendency to aggregate and to adsorb to various surfaces (1, 9). The molecule contains at least one reactive thiol group and tends to form disulfide-linked oligomers (9). Its tendency to bind to various tissues may be a phenomenon related to these properties. Further studies of the tissue distribution of vitronectin and of its interactions with elastin, elastotic material, amyloid, SAP, fibrillin and NKH-1 will be helpful in elucidating the physiological role of vitronectin.

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