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Immunohistochemical Studies on Fibrillin in Amyloidosis, Lichen ruber planus and Porphyria

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The pathogenesis of macular amyloidosis and lichen amyloidosis remains unsolved and the primary amyloid fibril protein(s) has not yet been identified. Ultrastructural association of skin amyloid with elastin associated microfibrils has been noted earlier. The presence of fibrillin in conjunction with such microfibrils was recently demonstrated immunohistochemically. The presence of fibrillin immunoreactivity in the amyloid deposits in skin biopsies from 3 patients with macular amyloidosis and 3 patients with lichen amyloidosis was studied, using monoclonal anti-fibrillin antibodies. For comparison, skin specimens were studied from five patients with lichen ruber planus, four patients with erythropoietic protoporphyria and from a patient with myeloma-associated cutaneous amyloidosis. Renal specimens from two cases of the amyloid A type of renal amyloidosis also were investigated.

There was no immunostaining either of the keratin bodies in specimens of lichen ruber planus, the cutaneous PAS-positive vascular deposits in patients with erythropoietic protoporphyria, or the amyloid deposits in specimens of systemic amyloidosis and it was faint or absent in amyloid deposits in the specimens from patients with lichen amyloidosis. In contrast, distinct fibrillin immunoreactivity could be demonstrated in amyloid deposits in specimens from patients with macular amyloidosis. It was sometimes absent in deposits located in the upper part of the papillary dermis, close to the dermal epidermal junction zone, while consistently strong in deposits located lower down in the dermis. The results suggest that fibrillin or part of the fibrillin molecule may be present in some of the amyloid deposits in specimens of macular amyloidosis. ***Key words: Fibrillin; Amyloidosis; Lichen ruber planus; Erythropoietic protoporphyria.***

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Amyloid deposits may occur in the skin and in several other organs in systemic amyloidosis (1). In both types of primary localized cutaneous amyloidosis, lichen amyloidosis and macular amyloidosis, the amyloid deposits are confined to the skin (2, 3). Amyloid deposits consist of microfibrils with a typical ultrastructural configuration. In contrast to several other types of amyloidosis, it has not yet been possible to identify the amyloid fibril protein in the deposits of lichen amyloidosis or macular amyloidosis, due to difficulties involved in purifying the dermal amyloid (3). A variety of different cell types such as keratinocytes, fibroblasts, mastcells and melanocytes have been considered as possible synthesizers of amyloid in these disorders. Electronmicroscopy findings of keratinocytes forming electron-dense masses in the upper dermis provided the basis of the theory that keratinocytes undergo filamentous degeneraton to amyloid, much as keratin bodies are presumably formed by keratin filaments (4, 5, 6). However, other investigators have reported the absence of anti-keratin immunostaining of amyloid in primary cutaneous localized amyloidosis (7, 8).

Immunoreactivity both of serum amyloid P component (SAP) and vitronectin, present at the periphery of elastic fibers in normal skin in adults, has been demonstrated in association with amyloid deposits of systemic amyloidosis, macular amyloidosis and lichen amyloidosis (2, 9–14). Fibrillin was recently demonstrated immunohistochemically to be present in conjunction with elastin associated microfibrils and other 8–10 nm microfibrils in a unique dermal network, and is presumably a primary structural constituent of such microfibrils (15, 16).

The present study was prompted by several reports of an ultrastructural association of amyloid and elastic fibers in various forms of skin amyloidosis, the aim being to investigate, using immunohistochemical methods, whether fibrillin is related with the cutaneous amyloid deposits in macular amyloidosis and lichen amyloidosis (17–19). For compari-

Table I. *Immunoreactivities of fibrillin, vitronectin, serum amyloid P component (SAP) and amyloid A component (AA) in amyloid deposits in different types of amyloidosis, and in keratin bodies and PAS positive vascular deposits in erythropoietic protoporphyria*

	Anti-fibrillin	Anti-vitronectin	Anti-SAP	Anti-AA
Macular amyloidosis	+	+	+	−
Lichen amyloidosis	−(+)	+	+	−
Systemic amyloidosis				
AA type	−	+	+	+
Light chain type	−	+	+	−
Keratin bodies	−	+	+	−
PAS-positive vascular deposits	−	+	+	−

son, specimens from patients with systemic amyloidosis were also studied. Because, as in amyloid, immunoreactivity of vitronectin and SAP is present in the PAS-positive vascular deposits in skin specimens from patients with erythropoietic protoporphyria and in keratin bodies in the skin of lichen ruber patients, skin specimens from such patients also were included in the study (6, 14, 20).

MATERIALS AND METHODS

Biological tissue

The specimens were obtained by biopsy of skin lesions from patients with *macular amyloidosis* (3 cases), *lichen amyloidosis* (3 cases), *cutaneous light chain type amyloidosis (1 case), lichen ruber planus* (5 cases) and *erythropoietic protoporphyria* (4 cases) the diagnoses having been based on clinical, histological and (when relevant) biochemical criteria. Patients with macular amyloidosis had pigmented macules in a rippled pattern on the upper part of the back, and those with the papular type of PLCA had typical firm, brown, smooth papules on the front of the lower legs. The specimens of erythropoietic protoporphyria skin and three of the specimens from patients with skin amyloidosis were surplus material from a previous study on vitronectin immunoreactivity as were two renal biopsy specimens obtained from patients with *systemic type A amyloid amyloidosis* and rheumatoid arthritis (13, 14).

Fixation procedure

Apart from a few that were frozen directly after collection, specimens were immersed in a transport medium (550 g ammoniumsulfate added to 1 liter 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 specimens were stored at −70°C. Cryostat sections, between 4 and 10 μm thick, were cut and fixed in acetone for 20 min at 4°C, consecutive sections being treated with antibodies or histochemical stain (see below).

Immunohistochemical techniques

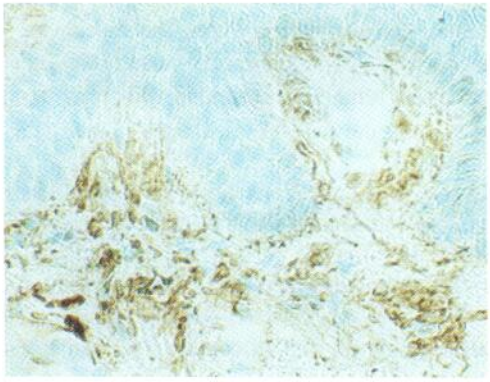
Both the *avidin-biotin-peroxidase complex technique* and the *alkaline phosphatase anti-alkaline phosphatase* (APAAP) complex technique were used (21, 22). Biopsies

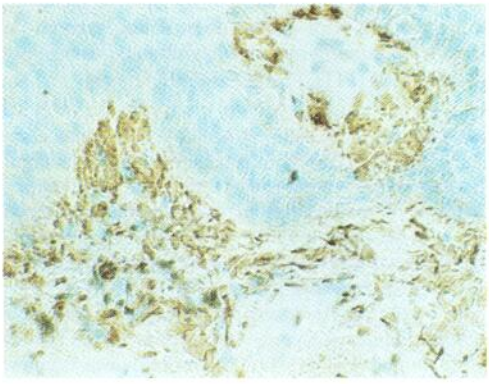
Fig. 1. Fibrillin immunoreactivity in amyloid deposits in *macular amyloidosis*. Staining of consecutive sections of a skin specimen from a patient with macular amyloidosis, using anti-fibrillin (a) and anti-vitronectin (b) in the avidin biotin peroxidase complex technique, demonstrating fibrillin immunoreactivity of amyloid deposits in the papillary dermis and the upper reticular dermis (×165).

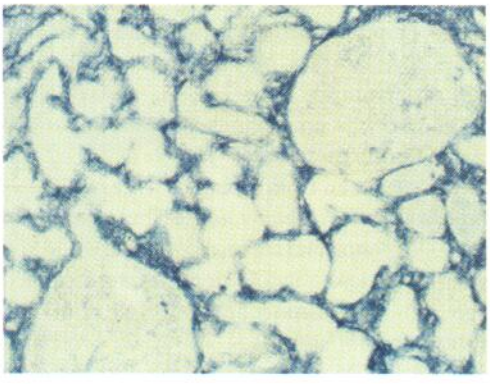
Fig. 2. Absence of fibrillin immunoreactivity in amyloid deposits of *systemic renal amyloidosis*. Section of a renal specimen from a patient with type AA amyloidosis sequentially stained with monoclonal anti-fibrillin in the APAAP technique (a) and with polyclonal anti-AA antibodies in an avidin biotin peroxidase-complex technique (b), demonstrating the absence of fibrillin immunoreactivity in the deposits of type A amyloidosis. There is a distinctly fibrillin immunoreactive peritubular network, also present in normal kidneys (unpublished observation) (×165).

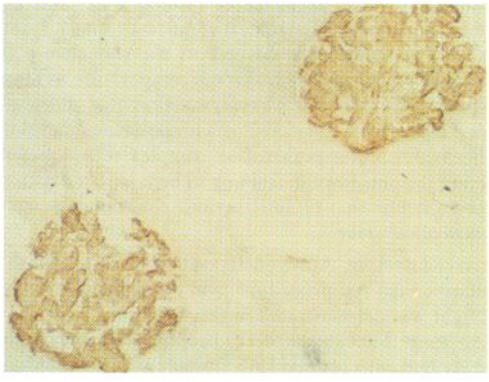
Fig. 3. Comparison of the distribution of fibrillin and vitronectin immunoreactivities in specimens of *lichen ruber planus*. Section of a skin specimen from a patient with lichen ruber planus sequentially stained with monoclonal anti-fibrillin in an avidin biotin peroxidase complex technique (a) and polyclonal anti-vitronectin and FITC conjugated swine anti rabbit IgG antibodies in an immunofluorescence technique (b), demonstrating keratin bodies that lack fibrillin immunoreactivity. Epidermis is seen to the left. The arrows in Fig. b point at some keratin bodies and colocalize with corresponding arrows in Fig. a. Note the presence in the papillary dermis of a network of thin fibrillin reactive fibers, not stained by anti-vitronectin (×165).

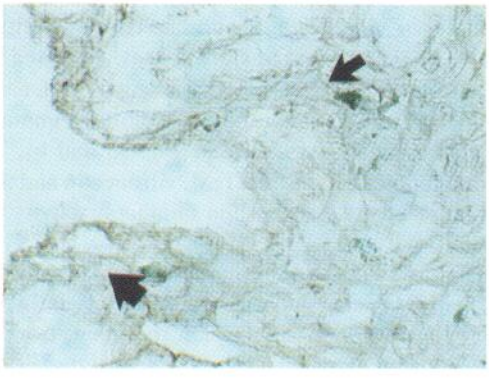
Fig. 4. Absence of fibrillin immunoreactivity in abnormal cutaneous vascular deposits in *erythropoietic protoporphyria*. Sections of a skin specimen from a patient with erythropoietic protoporphyria, immunostained with anti-fibrillin (a) and anti-vitronectin (b) in an avidin biotin peroxidase complex technique, demonstrating lack of fibrillin immunoreactivity in cutaneous vascular walls, stained by anti-vitronectin. There are elastotic changes in the surrounding tissue (×100).

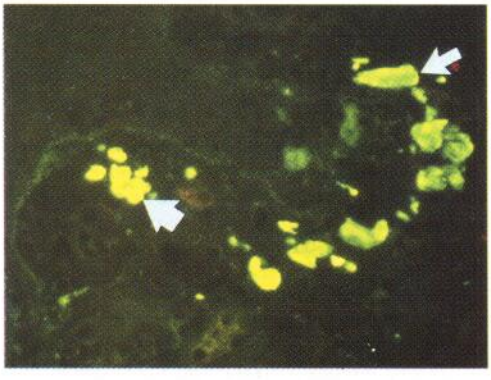


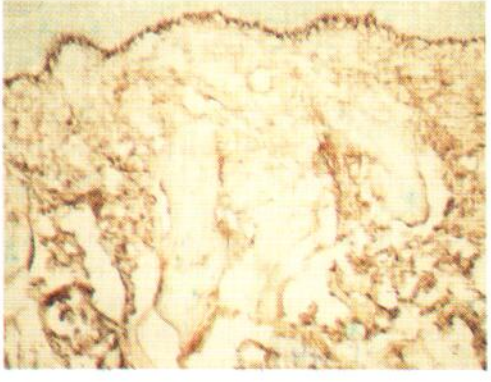












from the patients with lichen ruber planus were also analysed with *immunofluorescence* techniques, using fluorescein-conjugated (FITC) rabbit antihuman IgM.

In a *double staining technique*, the specimens from lichen planus patients were stained first by polyclonal anti-vitronectin with FITC swine anti-rabbit IgG as secondary antibodies. After photography, the coverslips were removed and the same sections were stained in the avidin biotin peroxidase complex technique using monoclonal anti-fibrillin with biotinylated horse anti-mouse IgG antibodies as secondary antibodies.

Some of the amyloidosis specimens were studied with *sequential staining procedures* as follows. In the first reaction, the monoclonal anti-fibrillin antibody was used in the APAAP complex technique, producing a blue color on the immunreactive structures: after photography, the slides were left in 50 mM Tris-HCL, 0.15 M NaCl, pH 7.5, for 12 h at 37°C to detach the coverslips; the slides were then treated with xylol for 3 min at room temperature to remove the blue color. In the second reaction the slides were stained either with congo red or with polyclonal antibodies in the avidin biotin perioxidase complex technique, thus enabling the anti-fibrillin staining to be compared with that of congo red or that of anti-amyloid A antibodies.

Histochemical stains

Congo red staining: Amyloid deposits were stained with the standard congo red procedure, and birefringence in polarized light was demonstrated in the amyloidosis specimens.

PAS stain: Specimens of erythropoietic protoporphyria were stained with routine PAS staining, demonstrating abnormally thick PAS-positive cutaneous vessel walls.

Methyl green was used to visualize epidermal and dermal cells on sections stained by the avidin biotin peroxidase complex technique.

Proteins and primary antisera

Monoclonal anti-fibrillin was produced by Sakai et al. (15). The working dilution was 1:3000. Polyclonal anti-vitronectin antibodies (as earlier characterized) were used at a working dilution of 1:10000 (avidin biotin peroxidase complex technique) or 1:1000 (immunofluroescence technique) (11–14). The sequence specific antibodies against amyloid A (AA) were a gift of Dr A Grubb, Department of Clinical Chemistry, Malmö General Hospital, Malmö, Sweden (23). The working dilution was 1:50. Antibodies against SAP (working dilution 1:10000) and FITC rabbit anti-human IgM (working dilution 1:50) were purchased from Dakopatts.

RESULTS

The intensity of immunostaining produced by the various antibodies differed from one type of deposit to another (Table I).

Distinct fibrillin immunostaining of amyloid deposits in the papillary dermis was demonstrated in the three specimens from patients with *macular amyloidosis*. These deposits were also stainable with congo red, anti-vitronectin and anti-SAP, but not with anti-AA antiserum. The intensity of fibrillin immunoreactivity varied within the specimens, and deposits in the papillary dermis close to the junction zone often remained unstained, whereas deposits lower in the dermis were distinctly stained (Fig. 1a and b).

In the specimens from the three patients with *lichen amyloidosis*, the fibrillin immunoreactivity was faint or absent in the amyloid deposits (not shown).

In the specimens of *systemic renal AA amyloidosis*, there was amorphous, congophilic material in several glomeruli and in some vessel walls. These deposits had distinct immunoreactivity with anti-AA, anti-SAP and anti-vitronectin (Fig. 2b). Fibrillin immunoreactivity was found as in normal kidneys in a fibrillar peritubular network (unpublished observation). However, there was no immunostaining of the amorphous amyloid deposits with the anti-fibrillin antibodies (Fig. 2a).

In the specimen from a patient with *systemic myeloma-associated skin amyloidosis* there were congophilic amorphous masses in the papillary and reticular dermis. These amyloid deposits were stained by congo red, anti-vitronectin, anti-SAP, but not by anti-fibrillin (not shown).

Keratin bodies were present close to the dermal-epidermal junction in the specimens from patients with *lichen ruber*, and a band-like cellular infiltrate was to be seen in the papillary dermis. This area had no vitronectin or SAP immunoreactivity other than that of keratin bodies, though a fibrillin immunoreactive network was present (Fig. 3a and b). The keratin bodies had distinct IgM, vitronectin and SAP immunreactivity, but fibrillin immunoreactivity was absent.

There was no fibrillin immunostaining of the abnormally vascular deposits, stainable by anti-vitronectin and anti-SAP, in the skin specimens from the patients with *erythropoietic protoporphyria* (Fig. 4a and b).

DISCUSSION

This study was prompted by the recent identification of fibrillin, a presumptive structural component of 8–12 nm microfibrils, and the production of monoclonal anti-fibrillin antibodies.

The results presented here indicate that fibrillin, or part of the fibrillin molecule, may be present in the amyloid deposits in skin of patients with macular amyloidosis. The finding that fibrillin immunoreactivity is absent from renal and cutaneous systemic

amyloid deposits, abnormal vascular deposits of erythropoietic protoporphyria and keratin bodies, suggests that its presence in the deposits of macular amyloidosis is specific for this type of amyloid. In contrast, SAP and vitronectin are present in several types of pathological protein deposits, including several types of amyloid. However, the fibrillin immunostaining was faint or absent in some parts of the amyloid deposits in the macular amyloidosis specimens, especially in deposits close to the dermal epidermal junction zone. This may be due to actual lack of fibrillin in part of the deposits unless there is 'masking' of antigenic epitopes.

The absence of fibrillin immunoreactivity in keratin bodies, indicates that there may be different mechanisms involved in the formation of keratin bodies and of amyloid deposits in macular amyloids. The faint or absent immunostaining by anti-fibrillin in the amyloid deposits of lichen amyloidosis indicates that it may have a different pathogenesis than macular amyloidosis, as has been suggested earlier (24). Studies of purified amyloid derived from macular and lichen amyloidosis specimens are needed to elucidate its molecular composition and to clarify whether a relationship does in fact exist between the presence of fibrillin and that of such amyloid deposits.

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REFERENCES

1. Westermark P. Occurrence of amyloid deposits in the skin in secondary systemic amyloidosis. Acta Pathol Microbiol Scand 1972; Section A 80: 718.
2. Breathnach SM. Amyloid and amyloidosis. J Am Acad Dermatol 1988; 18: 1–16.
3. Wong C-K. Review Cutaneous Amyloidosis. Int J Dermatol 1987; 26: 273–277.
4. Kobayashi H, Hashimoto K. Amyloidogenesis in organ-limited cutaneous amyloidosis: An antigenic identity between epidermal keratin and skin amyloid. J Invest Dermatol 1983; 80: 66–72.
5. Hashimoto K. Progress on cutaneous amyloidoses. J Invest Dermatol 1984; 82: 1–3.
6. Linser I, Auböck J, Romani N, Smolle J, Grubauer G, Fritsch P, Hintner H. The keratin body phenomenon. Epithelia 1987: 85–99.
7. Ishii M, Asia Y, Hamada T. Evaluation of cutaneous amyloid employing anti-keratin antibodies and the immunoperoxidase technique (PAP method). Acta Derm Venereol (Stockh) 1984; 64: 281–285.
8. Norén P, Westermark P, Cornwell GG, Murdoch W. Immunofluorescence and histochemical studies of localized cutaneous amyloidosis Br J Dermatol 1983; 108: 277–285.
9. Breathnach SM, Bhogal B, Dyck RF, de Beer FC, Black MM, Pepys MB. Immunohistochemical demonstration of amyloid P component in skin of normal subjects and patients with cutaneous amyloidosis. Br J Dermatol 1981; 105: 115–124.
10. Breathnach SM, Melrose SM, Bhogal B, de Beer FC, Dyck RF, Black MM, Tennent G, Pepys MB. Amyloid P component is located on elastic fibre microfibrils in normal human tissue. Nature 1981; 293: 652–254.
11. Dahlbäck K, Löfberg H, Dahlbäck B. Localization of vitronectin (S-protein of complement) in normal human skin. Acta Derm Venereol (Stockh) 1986; 66: 461–467.
12. Dahlbäck K, Löfberg H, Alumets J, Dahlbäck B. Immunohistochemical demonstration of age-related deposition of vitronectin (S-protein of complement) and terminal complement complex on dermal elastic fibers. J Invest Dermatol 1989; 92: 727–733.
13. Dahlbäck K, Löfberg H, Dahlbäck B. Immunohistochemical demonstration of vitronectin in association with elastin and amyloid deposits in human kidney. Histochemistry 1987; 87: 511–515.
14. Dahlbäck K, Löfberg H, Dahlbäck B. Immunohistochemical studies of vitronectin in elastic tissue disorders, cutaneous amyloidosis, lichen ruber planus and porphyria. Acta Derm Venereol (Stock) 1988; 68: 107–115.
15. Sakai LY, Keene DR, Engvall E. Fibrillin A New 350-kD Glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986; 103: 2499–2509.
16. Dahlbäck K, Ljungquist A, Löfberg H, Dahlbäck B, Engvall E, Sakai L. Fibrillin immunoreactive fibers constitute a unique network in the human dermis. J Invest Dermatol. In press.
17. Danielsen L, Kobayasi T. An ultrastructural study of cutaneous amyloidosis. Acta Derm Venereol (Stockh) 1973; 53: 13–21.
18. Kobayasi T, Asboe-Hanson G. Ultrastructure of skin in primary systemic amyloidosis. Acta Derm Venereol (Stockh) 1979; 59: 407–413.
19. Yanagihara M, Kato F, Shikano Y, Fukushima N, Mori S. Intimate structural association of amyloid and elastic fibers in systemic and cutaneous amyloidosis. J Cutan Pathol 1985; 12: 110–116.
20. Breathnach SM, Bhogal B, De Beer FC, Melrose SM, Black MM, Pepys MB. Immunohistochemical studies of amyloid P component and fibronectin in erythropoietic protoporphyria. Br J Dermatol 1983; 108: 267–275.
21. Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981; 75: 734–738.

22. Cordell JL, Falini B, Erber WN, et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984; 32: 219–229.
23. Grubb A, Löfberg H, Thysell H, et al. Production of an aminoacid sequence-specific antiserum against human amyloid A (AA) and serum amyloid A (SAA) protein. Scand J Clin Lab Invest 1987; 47: 619–626.
24. Westermark P, Noren P. Two different pathogenetic pathways in lichen amyloidosus and macular amyloidosis. Arch Dermatol Res 1986; 278: 206–213.