Immunochemistry 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 conjuction 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 absent 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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Table 1. 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

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<thead>
<tr>
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<th>Anti-fibrillin</th>
<th>Anti-vitronectin</th>
<th>Anti-SAP</th>
<th>Anti-AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macular amyloidosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lichen amyloidosis</td>
<td>(−/+)</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Systemic amyloidosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AA type</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Light chain type</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Keratin bodies</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>PAS-positive vascular deposits</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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</table>

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 issue

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-ethylmaleamide, 5 mM magnesium sulfate) being washed within 48 h in transport medium lacking ammonium sulfate and then immediately frozen in chlorofluoromethane 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 (AAPAP) 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 AAPAP 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 AA 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 elastic 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 amyloidotic 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 immunoreactive structures; after photography, the slides were rehydrated 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 xylene 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 peroxidase 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 amyloidotic specimens.

PAS stain: Specimens of erythropoietic protoporphyrinia 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 1:5000. Polyclonal anti-vitronectin antibodies (as earlier characterized) were used at a working dilution of 1:1000 (avidin biotin peroxidase complex technique) or 1:1000 (immunofluorescence technique) (11–14). The sequence specific antibodies against amyloid A (AA) were a gift of Dr A Grubh, 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 immunoreactivity, 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 protoporphyrinia (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 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 amyloidosis. 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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