Nature of Amyloid Deposits in Hypernephroma

Immunocytochemical Studies in 2 Cases Associated With Amyloid Polyneuropathy

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Two patients who presented with amyloid polyneuropathy were found to have an amyloid-positive hypernephroma. The amyloid extracted from the tumor of one patient was purified by gel filtration and found to immunoreact by immunodiffusion, only with antisera against denatured lambda-type amyloid protein but not with antisera against denatured x amyloid, AA, or prealbumin. With the unlabeled immunoperoxidase method or immunofluorescence in combination with specific antisera, it was shown that in both patients the amyloid deposits in the tumor, kidney, lymph node, muscle, and nerve had lambda-type amyloid antigenic fibril determinants. Some regions, amyloid-negative by congo red, immunoreacted with anti-lambda antiserum and were shown to represent amyloid fibrils electron microscopically. Several plasma cells found in the tumor and lymph node immunoreacted specifically with the anti-amyloid lambda antiserum. The findings provide the first observation that the amyloid in hypernephroma can be of immunocytic origin, even in the absence of overt signs of plasma cell dyscrasias, and suggest that amyloid polyneuropathy could be the presenting sign of hypernephroma. (Am J Pathol 1984, 116:447–454)

IN MALIGNANCIES, systemic, nonlocalized amyloidosis most frequently occurs with immunocytic dyscrasias and only rarely with true carcinomas.1-5 Excluding the tumors associated with localized amyloid, the incidence of generalized amyloidosis in patients with cancer has been estimated to be between 0.1 and 0.4% among all cancers.1-2 From all cancerous tumors, hypernephroma appears to be an important exception, because these tumors are responsible for 25-33% of all cancers associated with amyloidosis.5-7 This is highly significant when we take into account that renal carcinomas constitute only 2-3% of all carcinomas in studies that do not exclude immunocyte-derived tumors.5-8 The factors predisposing some patients with tumor, especially renal cell carcinoma, to the development of amyloidosis, as well as the precursor protein responsible for the formation of this amyloid, are unknown.

Recent advances in biochemical and immunologic analysis of amyloid fibril proteins in systemic amyloidosis have disclosed at least three different types of amyloid fibril proteins and their precursors.3 Immunoglobulin light chain-derived protein (AL) comprises the amyloid fibrils of the immunocytic ("primary") diseases,9 protein A comprises the amyloid fibrils of the reactive ("secondary") AA type, associated with chronic inflammatory conditions,10,11 and a variant of prealbumin comprises the major component of amyloid in hereditary amyloid polyneuropathy.12-14

The chemical nature of amyloid deposits in hypernephroma and its precursor protein have not been precisely ascertained, but it has been suggested that the neoplasm itself is the most probable causative factor.8 In analogy to the amyloid associated with Hodgkin's disease8 and on the basis of organ involvement,6-8,14 it has been suggested that the amyloid in hypernephroma is probably of the reactive, "sec-

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ondary,” AA type. In one such case it was found that this amyloid may contain AA amyloid fibril protein.17

We report here two patients with amyloid-positive hypernephroma who presented with typical signs of acquired amyloid polyneuropathy.13,18-20 Evidence is provided that the amyloid extracted from the tumor was of lambda (A\(\lambda\)) immunoglobulin origin and that the amyloid deposits in several tissues recognized only A\(\lambda\) antigenic fibril determinants.

Materials and Methods

Summary of Case Reports

Case 1

A 67-year-old man presented with slowly progressive, predominantly distal, muscle weakness and diminished sensation of 6–7 years’ duration. Four years prior, he had had a left hypernephroma removed. His physical examination was normal. Specifically, he had no organomegalies or lymphadenopathy and did not complain of weight loss or anorexia. Neurologic examination showed some fasciculations and signs of sensorimotor neuropathy with an early autonomic component. Electromyography was consistent with an axonal neuropathy. Serum immunoglobulins and immunoelectrophoresis were normal. Bone marrow aspirate was normocellular. Urine was negative for Bence Jones proteinuria. Muscle biopsy showed signs of denervation11; with crystal violet and Congo red staining, deposits of amyloid were seen in intramuscular blood vessels (Figure 3A); the connective tissue surrounding muscle fibers (an area characteristically positive for amyloid in amyloid neuropathies)13-18 was negative for amyloid. Nerve biopsy showed fibrosis and decreased myelin but no amyloid deposits. Workup for malignancy revealed a vascular tumor in the right kidney without signs of metastasis. The excised tumor was a hypernephroma composed mainly of clear cells and occasional scattered granular cells. Amyloid was present in the interstitial tissue of the tumor and in the vessels, glomeruli, and medulla of the surrounding kidney.

The patient’s condition progressively worsened. The neuropathy caused him severe autonomic and sensorimotor disability, and within the next 2 years metastatic lesions were found in lungs and liver.

Case 2

This 67-year-old man presented with distal paresthesias and painless hematuria. A tumor, found in the left kidney, was excised and was shown to be a hypernephroma with deposition of amyloid in the tumor and in glomeruli and vessels of the surrounding renal tissue. The patient’s initially minor sensory complaints rapidly progressed to a severe painful sensorimotor neuropathy characterized by symmetrical muscle weakness and wasting, decreased distal sensation, and absent reflexes. Electromyography confirmed the diagnosis of a severe axonal neuropathy. Autonomic dysfunction with orthostatic hypotension, diarrhea, and impotence soon developed. Further workup showed a five-fold elevated serum IgM with an IgM lambda spike on immunoelectrophoresis, mild anemia, an unremarkable bone marrow examination, and factor X deficiency. Muscle biopsy showed denervation11 with amyloid deposits in both vessels and perimysium (Figure 4A); nerve biopsy showed fibrosis with decreased myelin and amyloid deposits in the endoneurial parenchyma. Involvement of the heart and liver by amyloid was suspected because of diphosphonate scans22 and a cardiac echocardiogram. No metastatic lesions were found in liver, spleen, lung, or bones. Urine was positive for Bence Jones proteinuria. The repeat bone marrow biopsy specimen was essentially normal, although slightly hypocellular and with mild plasmacytosis; some vessels in the bone marrow biopsy were amyloid-positive with Congo red staining.

Isolation of Amyloid Fibril Protein

Amyloid fibrils were isolated from 4.7 g of frozen amyloid-laden renal tumor from the first patient (Case 1) by repeated homogenization in phosphate-buffered saline. Supernatant was examined for AA amyloid protein by radioimmunoassay, as previously described.23 The residue was subsequently homogenized in distilled water, and 79 mg of crude amyloid fibrils were recovered by lyophilization of the water suspension and the top layer of the sediment, as previously described.24,25

Purification of Amyloid Fibrils Protein and Immunologic Studies

The lyophilized fibrils were solubilized in 6 M guanidine-HCl with 0.1 M Tris buffer, pH 8.2. This “crude” extract was chromatographed on a Sephadex G-100 (1.6 × 100 cm) column equilibrated in 5 M guanidine HCl. Fractions of each peak (V0, “shoulder,” Pk) (Figure 1) were dialyzed in distilled water and lyophilized. Double immunodiffusion was used for immunologic characterization of the 6 M guanidine-denatured but unfractonated (crude) fibril and the purified amyloid proteins of each column fraction. The antisera used were against human prealbumin (Accurate Chemical) and against amyloid
proteins AA, amyloid of lambda type (Aλ) and amyloid of kappa type (Ax) obtained by immunizing rabbits with 6 M guanidine-denatured amyloid fibril proteins of each type and characterized as previously described. Briefly, these antisera were characterized by immunodiffusion as follows: the antiserum to AA showed a single precipitation line between purified AA and no lines between normal human serum, 6 M guanidine-denatured human spleen, amyloid lambda and kappa proteins, or amyloid P components. Anti-Aλ reacted with at least four Aλ proteins from other patients and some lambda type Bence Jones proteins but failed to react with Ax, AA, or P component. Anti-Ax reacted with homologous Ax proteins but failed to react with Aλ, AA, or P component. Anti-Aλ and anti-Ax were further absorbed with normal human serum and 6 M guanidine-denatured normal human spleen. Anti AA was purified by passage through an immuno-absorbent column of AA agarose. Antiserum against human prealbumin was characterized as previously described.

SDS–Polyacrylamide Gel Electrophoresis

SDS–polyacrylamide gel electrophoresis of the purified amyloid proteins of each column fraction was performed as previously described. For molecular weight determinations, ovalbumin, chymotrypsinogen A, cytochrome C, and insulin were used as molecular weight markers.

**Histologic, Immunocytochemical, and Electron-Microscopic Studies**

Frozen and paraffin-embedded sections of the tumor, muscle, and nerve biopsies from both patients were examined with hematoxylin and eosin (H & E) and crystal violet or Congo red stain for amyloid. Muscle biopsy from the first patient was also processed for electron microscopy. We performed immunocytochemical studies on frozen sections using anti-AA, anti-Aλ, anti Ax, and anti-prealbumin as primary antisera, and stained with FITC-goat anti-rabbit IgG(Fab′)2 fragment (Cappel Laboratories) in an indirect immunofluorescence technique, as previously described. Paraffin sections from the tumors and muscle biopsies were also stained with the above antiserum and a peroxidase-antiperoxidase (PAP) technique, as previously described. Appropriate control studies were used as described previously.

**Results**

The supernatant, obtained during the isolation of amyloid fibril from the tumor of the first patient, was negative for AA antigenicity, as screened by radioimmunoassay. The elution profile, obtained when isolated and dissociated amyloid was gel-filtered on Sephadex G-100, is shown in Figure 1. In addition to the void volume, Vo, two other protein fractions, “shoulder” and Pk, were eluted. The antigenic properties of the “crude” tumor amyloid and its three eluted subfractions, as determined by immunodiffusion, are summarized in Table 1. Both “crude” and “shoulder” fractions reacted only with anti-Aλ and not with anti Ax, anti-AA, or anti-prealbumin. A reaction of antigenic identity, as shown in Figure 2, clearly indicates that the amyloid from hypernephroma shares antigenic determinants only with Aλ. SDS–polyacrylamide gel electrophoresis showed that the Aλ-reactive fraction (“shoulder”) was com-

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Table 1—Immunodiffusion of the Extracted Amyloid Using Antisera Against Denatured Amyloid Proteins

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>“Crude”</th>
<th>Vo</th>
<th>“Shoulder”</th>
<th>Pk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-AA</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anti-Aλ</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Anti-Ax</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anti-prealbumin</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

+, positive immunoreaction, −, negative immunoreaction.
posed of two bands in the migration of estimated molecular weight between 12,500 and 30,000; Pk, which had no antigenicity with any of the used antisera, had an estimated molecular weight of 8000.

In several sections of the muscle biopsy of the Patient 1, amyloid was present only in 1–2 vessels (Figure 3A) and not in the areas surrounding muscle fibers, as is usually the case in amyloid neuropathy13,18 and as found in Patient 2 (Figure 4A); anti-\( \alpha \) antiserum, however, stained in the muscle several Congo red or crystal violet-negative regions (Figure 3B) in a pattern identical to that seen with crystal violet in patients with amyloid neuropathies3,18 and similar to that found in Patient 2 (Figure 4A). In several areas around muscle fibers of the same biopsy (which were amyloid-negative by Congo red but had \( \alpha \)-positive regions by immunofluorescence), we found electron-microscopically deposits of amyloid fibrillar proteins (Figure 3C). Further immunocytochemical studies also confirmed that the amyloid deposits in the renal tumor immunoreacted only with the anti-\( \alpha \) antiserum and not with the \( \alpha \)x, AA, or prealbumin.

The muscle biopsy of the second patient clearly showed amyloid deposits around muscle fibers (Figure 4A). The amyloid deposited in muscle, nerve, tumor, and surrounding kidney reacted immunocytochemically only with anti-A\( \alpha \) antiserum and not with anti-\( \alpha \)x, AA, or prealbumin (Figure 4B and C). Within the tumor (Figure 4D) and adjacent kidney (Figure 4C) some lymphoid infiltrates and plasma cells were present and found to immunoreact with anti-A\( \alpha \) antiserum. An excised lymph node found next to the tumor had several A\( \alpha \)-positive plasma cells (Figure 4E). In serial sections (Figure 4F and 4G) areas amyloid-positive by crystal violet (Figure 4F) were identically stained with antiamyloid A\( \alpha \) antiserum (Figure 4G).

Discussion

Two patients who presented with amyloid polyneuropathy were found to have an amyloid-positive hypernephroma. The amyloid proteins extracted from the renal tumor of one patient who had no overt signs of plasma cell dyscrasia had molecular weight within the range of the immunoglobulin light chain and immunoreacted only with antiserum against lambda amyloid light chain (A\( \lambda \)). In both patients, the amyloid deposited in the renal tumor and other tissues immunoreacted exclusively with the A\( \lambda \) antiserum and not with antiserum raised against the other amyloid proteins (AA, A\( \alpha \)) or prealbumin. These findings suggest that the amyloid associated with hypernephroma is of immunocytic origin and appears to be related to a lambda light polypeptide chain. These precursor light chains have been systemically disseminated in view of the presence in both patients of a severe symmetrical sensorimotor and autonomic amyloid polyneuropathy.

The amyloid associated with hypernephroma has been thought to be of the “reactive,” secondary, AA type.1,2,8,17,31 Indeed, amino acid sequence analysis of splenic amyloid fibril deposits in a case of hypernephroma isolated by an acid extraction method revealed homology with AA protein.31 This extraction method would not, however, solubilize an AL protein if present.3,25,40 Our conclusions are, therefore, that this case may represent an inflammation-associated AA type of fibril deposition secondary to renal infection rather than fibril deposition directly caused by the tumor; or 2) that the latter case may represent a mixture of AL and AA proteins, of which only the AA was demonstrated; or 3) that hypernephromas may be associated with either pure AA or AL proteins. Detailed review of the other previously reported cases discloses an increased frequency of plasma cell proliferation or circulating abnormal immunoglobulins in several of these patients. Kiely has pointed out that hypernephroma is the tumor that can cause the most pronounced plasmacytic response in the bone marrow.32 Among several patients with hypernephroma and amyloid reviewed from the literature, we noted that 4 patients had an increased number of plasma cells in their bone marrow associated with diffuse hypergammaglobulinemia,8,33–35 3 patients had Bence Jones proteinuria,2,8,32 and 2 patients had osteolytic spots in the skull resembling myelomatosis.8,34 These observations are consistent with our present study where a circulating IgM\( \lambda \) monoclonal immunoglobulin was found in 1 patient, and several plasma cells positive for A\( \lambda \) immunoglobulin were present in the tumor. These findings, along with the immunoreactivity of the amyloid in both patients with antiserum to A\( \lambda \) amyloid protein, further indicate that in patients with hypernephroma there may exist a plasma cell dyscrasia (often occult), and the amyloid, if present, can be of immunocytic origin.
Although bone marrow cells were not stained for \( x \) and \( \lambda \) light chains to unequivocally prove their monoclonality, the observed immunoreactivity of the lymph node cells only with anti-\( \lambda \) antiserum and not with anti-\( \alpha \)-\( \lambda \) (Figure 4E) suggests that the lymphoid cells were monoclonal for the specific production of amyloidogenic \( \lambda \) light chains.

The site of production of the amyloid in patients with hypernephroma is uncertain. Its precursor \( \alpha \)-\( \lambda \) protein may have originated in the tumor, as suggested by the \( \alpha \)-\( \lambda \)-positive plasma cells found in juxtaposition to the amyloid, or in the lymphoid organs, as suggested by the \( \lambda \)-positive lymphoid cells found in the neighborhood of the tumor lymph node, or in both. The local production of amyloid light chains by cells in the tumor may have been facilitated by the chronic inflammation that accompanies hypernephroma, resulting in influx in the tumor and the surrounding renal tissue of lymphoplasmacellular elements, one of which may have become monoclonal. Like the antibody-producing cells in the lymph nodes and bone marrow, this monoclonal can...
Figure 4 - Tissues from Patient 2.  

A - Muscle biopsy stained with crystal violet has amyloid deposits in the perimysium surrounding several muscle fibers. (x 480)  

B - Amyloid in some glomeruli surrounding the tumor stained with Congo red shows yellow-green birefringence under polarized light. (Congo red and H&E, x 400)  

C - A section from the same tissue stained with anti-\( \alpha \)-antiserum and PAP shows that the amyloid deposits in the glomeruli immunoreacted with anti-\( \alpha \). Occasional plasma cells are \( \alpha \)-positive. (x 460)  

D - A section of the tumor stained with anti-amyloid lamda antiserum and PAP, reveals \( \alpha \)-positive areas and several scattered \( \alpha \)-positive plasma cells. (x 460)  

E - A section of a lymph node, adjacent to the tumor, stained with anti-\( \alpha \) and PAP, reveals plasma cells with cytoplasm positive for \( \alpha \). (x 750)  

F - Serial sections of an amyloid-positive vessel next to the tumor, stained with crystal violet (F) and identically with anti-\( \alpha \) antiserum (G). (x 750)
proliferate and secrete an aberrant type of light polypeptide chain, which may have become "amyloidogenic." This precursor light chain is then circulated; and according to its affinity for certain tissues, it is modified, perhaps by proteolysis,\textsuperscript{27} to form insoluble fibrils having a twisted $\beta$-pleated sheet structure.\textsuperscript{28} The cell processing the light polypeptide chain of the immunoglobulin protein to fibrils is believed to be of phagocytic origin.\textsuperscript{3}

The amyloid associated with hypernephroma has been always systemic,\textsuperscript{2,6,8,16} with special predilection for the basement membrane of the small and medium-sized arteries. In the muscle biopsy of the first patient, amyloid was found by crystal violet and Congo red only on the walls of 1-2 vessels and not in the connective tissue surrounding the muscle fibers, a location that is almost always amyloid-positive in patients with amyloid polynephropathy.\textsuperscript{13,18} Electron-microscopically, however, amyloid fibril deposits were identified in the periphery of some muscle fibers, and these areas were found to immunoreact with antisera to $\alpha\lambda$ amyloid protein when an adjacent piece from the same biopsy was immunostained and viewed by light microscopy (Figure 3A–C). This suggests that amyloid-related proteins can form fibrillar deposits with $\alpha\lambda$ antigenic determinants before they develop into a form that allows Congo red (or crystal violet) molecules to be inserted among the amyloid proteins to give the characteristic staining pattern visible by light microscopy. Immunocytochemistry, therefore, can be in some cases superior to conventional methods in detecting early amyloid deposits.

Although the amyloid protein of immunoglobulin origin, comprised of the variable fragments of the light chains,\textsuperscript{41} has individual (idiotypic) antigenic specificity, there is considerable antigenic similarity or even identity among amyloid fibril proteins of a given variable subgroup from different individuals.\textsuperscript{39} Furthermore, light chains with certain variable subgroups tend to be associated with amyloid more often than other light chains.\textsuperscript{39} These indicate that an antisera made against an amyloid protein of one particular variable subgroup often can be a suitable reagent for detection and typing of other amyloid proteins of the same variable subgroups. Our antisera against $\alpha\lambda$ protein that characterized the amyloid of the present cases has also reacted with at least four $\alpha\lambda$ amyloid fibril proteins from other patients with $\alpha\lambda$ amyloid\textsuperscript{26,28,30} and has successfully characterized the $\alpha\lambda$ nature of amyloid of genitourinary tract in 9 patients.\textsuperscript{30} A negative reaction with the $\alpha\lambda$ antisreum cannot, however, rule out the presence of $\alpha\lambda$ antigens in an $\alpha\lambda$ amyloid, as has been also previously suggested.\textsuperscript{28}

The finding that the amyloid in our patients who presented with amyloid polyneuropathy is of immunocytic origin is in accord with previous observations that acquired amyloid polyneuropathies occur only with immunocytic dyscrasias and not with secondary, AA, "reactive" amyloidosis.\textsuperscript{13,20} Because hypernephroma can often be a "silent tumor," its presence should be suspected in patients who present with acquired amyloid polyneuropathy, with or without overt signs of plasma cell dyscrasia. Polyneuropathy can occur as a remote effect of nonimmunocytic tumors\textsuperscript{46}; but, in contrast to the present cases, it is amyloid-negative and lacks an autonomic component.

The observation that the amyloid in hypernephroma can be—at least in some cases—of immunoglobulin light chain origin, even in the absence of overt plasma-cell dyscrasia, may be of practical significance in the long-term management of patients with these tumors. Perhaps chemotherapeutic agents used for the management of plasma cell dyscrasias (i.e., myeloma) may prove to be beneficial in preventing or reducing formation of amyloid deposits in some of these patients and may beneficially affect their outcome and regression of the tumor.

References

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