Amyloid A in Systemic Amyloidosis Associated with Cancer

Gunnar Husby, Gudmund Marhaug, and Knut Sletten

Department of Rheumatology, Institute of Clinical Medicine, University of Tromsø, 9000 Tromsø, Norway [G.H., G.M.] and Institute of Biochemistry, University of Oslo, Oslo, Norway [K.S.]

ABSTRACT

Amyloid fibrils from two cases of cancer-associated, systemic amyloidosis with renal cell carcinoma and mesothelioma as the respective underlying disorders were studied. The immunochemical studies suggested strongly that amyloid A comprised a principal fibril component in both cases of cancer-associated amyloidosis. This was definitively proven by amino acid sequence analyses, which revealed structural homology between a purified subcomponent of the amyloid fibrils from both of the two cases of cancer-associated amyloidosis and previously sequenced amyloid A proteins. The chemical composition of the amyloid fibrils from systemic amyloidosis associated with cancer thus corresponded to that seen in amyloidosis reactive to inflammatory diseases and Hodgkin's disease. Amyloid proteins of immunoglobulin light chain type, which are found associated with myelomatosis, macroglobulinemia, and idiopathic (primary) amyloidosis, were not found in the two amyloid preparations. Renal cell carcinoma appears to be an effective stimulator of amyloid formation, while only one case of amyloidosis associated with mesothelioma has been reported previously.

INTRODUCTION

The amyloid fibrils appear to be the unique and principal component of all forms of amyloid substance regardless of clinical type of amyloidosis (5). The fibrils are made up of protein, and recent investigations have made it possible to establish a chemical classification of amyloid based on the structural properties of the different amyloid fibril proteins and to correlate the chemical classes of amyloid to various clinical types of amyloidosis (13). Homogeneous immunoglobulin light chains or differently sized aminoterminal fragments thereof, AL (for nomenclature of amyloid, see Ref. 10), proteins comprise a major component of the fibrils in idiopathic (primary) systemic amyloidosis and amyloidosis associated with immunocyte dyscrasias (9, 11) whereas AA plays a similar role in cases of reactive (secondary) systemic amyloidosis (3, 13). A serum protein, SAA, is larger but otherwise identical to AA and is thought to be its precursor (for references, see 20). In addition, a calcitonin-like protein, presumably a product of the malignant cells, has been shown to be a component of the amyloid found locally in medullary carcinomas of the thyroid (28). Also, some other tumors contain local amyloid deposits (19), but the chemical nature of such deposits is not known.

Systemic amyloidosis is quite frequently associated with malignant neoplastic disorders, and immunocyte dyscrasias account for the majority of such cases (30, 32). Hodgkin's disease and renal cell carcinoma are other cancers sometimes associated with systemic amyloidosis with a reported prevalence of 4 and 3.2%, respectively (2), while solid neoplasms other than renal cell carcinomas are only sporadically found in association with amyloidosis.

Whereas amyloid associated with immunocyte dyscrasias is of the immunoglobulin class (9) and amyloid reactive to Hodgkin's disease is of the AA class (18) corresponding to that associated with a variety of long-standing inflammations (27), the chemical nature of amyloid fibrils associated with other cancers (i.e., solid tumors) has not been established (9). It was, therefore, believed to be of interest to investigate the structure of the amyloid fibrils in 2 cases of cancer-associated systemic amyloidosis, one with renal cell carcinoma and the other with mesothelioma as the underlying disorder.

MATERIALS AND METHODS

Source of Amyloid Fibrils. Case 1, E. L., was a 53-year-old female, who for 2 years had felt tired, lost weight, and had periods of fever. At examination, a large tumor was felt in the left side of the abdomen and the liver was enlarged. The patient had an elevated sedimentation rate, hematuria, and proteinuria. At laparotomy, a large tumor of the left kidney, adherent to the colon, spleen, and pancreas, was removed. Histological examination of the tumor revealed a renal carcinoma made up by clear and eosinophilic cells with a compact growth pattern and extensive necrosis. Amyloid was found mainly in the vascular framework of the tumor. Biopsies from the liver and rectum showed amylodosis. Postoperatively, she got an infected fistula from the colon to the left flank, and she died 3 months after the operation. Extensive amyloidosis of the liver, spleen, and the right kidney was found at autopsy. No tumor metastases were detected. She had not suffered any rheumatic, infectious, or other malignant disease possibly underlying her systemic amyloidosis. It was concluded, therefore, that the amyloid was reactive to renal carcinoma. Tissue from the right kidney was selected for extraction of amyloid.

Case 2, B. K., was a 15-year-old male, who had been observed for 1 year because of anemia, elevated erythrocyte sedimentation rate, and increasing hepatomegaly. A needle biopsy from the liver showed massive amyloidosis. Except for occasional episodes of allergic rhinitis without infectious complications, the young patient had been previously healthy, and no disease possibly underlying the patient's amyloidosis could be found. The patient was, therefore, diagnosed as having primary (idiopathic) amyloidosis. The following year, the patient developed nephrosis, severe anemia, diarrhea, nausea, gastrointestinal bleedings, and increasing hepatomegaly. He died after 2 years due to renal failure and hemorrhagic diathesis. At autopsy, massive amyloidosis was found in the liver (5620 g), spleen (450 g), retroperitoneal lymph nodes, kidneys, adrenals, intestines, and blood vessels. The clinical pattern of amyloidosis was consistent with that of the reactive form of the disease. The autopsy findings also included a retroperitoneal tumor, located close to but without direct connection with the pancreas. The tumor was oval, soft, necrotic, and measured 8 x 4 cm.
The microscopic appearance was consistent with that of a mesothelioma, which showed a monomorphic epithelial papillary histology. Amyloid was not present in the malignant tissue, except for small deposits in capsular vessel walls. No other location of malignant tissue was detected, and it was concluded that the tumor represented the primary cancer. The diagnosis was confirmed by histological reexamination of the tumor by an independent pathologist. The tumor was also examined by the Armed Forces Institute of Pathology and by the Canadian Tumor Registry without any definitive diagnosis being made. However, both agreed that mesothelioma was a probable diagnosis. The generalized amyloidosis was thought to be reactive to the tumor, since no history or signs of concomitant infectious or inflammatory disease was found. It was believed improbable that the previous episodes of allergic rhinitis could initiate the formation of systemic amyloid. It was noted that the regional lymph nodes were massively involved by amyloid. Liver tissue was used for extraction of amyloid.

**Extraction and Purification of Amyloid Fibril Proteins.** The amyloidotic tissues were cut into small pieces and subjected to extraction of amyloid fibrils with water after repeated washings of homogenized tissue with 0.9% NaCl solution (23). The fibril-containing water supernatants were lyophilized, treated with 0.6 M guanidine and a reducing agent, 0.05 M dithiothreitol, plus 1.0 mM EDTA, and thereafter subjected to gel filtration on Sephadex G-100 under dissociating conditions as described (12, 16). Selected protein fractions eluted from the Sephadex G-100 column were used for amino acid sequence studies after gel filtration on a Sephadex G-25 fine column (16).

**Electrophoresis.** The method for sodium dodecyl sulfate-polyacrylamide gel electrophoresis has been described by Swank and Munkres (31).

**Immunological Studies.** Double diffusion in 1% agarose gel was used for immunological characterization of crude DAM and purified amyloid proteins obtained by gel filtration (15, 16). In addition to DAM and protein fractions from amyloids E. L. and B. K., AA, SAA, and various amyloid proteins of AL type were used as controls (15, 20). Antisera to these proteins were also used in the immunodiffusion test system (15). Rabbit antibodies to human immunoglobulin κ- and λ-light chains (Bence-Jones proteins) were purchased from DAKO-immunoglobulins a/s, Copenhagen, Denmark.

**Structural Studies.** NH₂-terminal amino acid sequence analysis was performed by automated Edman degradation using the automatic sequence analyzer, JAS-47K, JEOL (27).

**RESULTS**

The yields of water-extracted amyloids from E. L. (kidney) and B. K. (liver) were 0.9 and 1.0 g, respectively, of lyophylized fibril material per 20 g of each of the fresh tissues. The fibril preparations exhibited the typical green birefringence of amyloid when stained with Congo red and examined microscopically under polarized light.

**Gel Filtration Studies.** The elution profiles obtained when dissociated and reduced amyloid E. L. and B. K. were gel filtered on Sephadex G-100 (Chart 1) were almost identical to each other and very similar to those of several reactive AA-type amyloids reported previously by us (14). In addition to a large protein peak eluted in the void volume (Chart 1A, V₀), approximately 45% of the protein material was eluted in a retarded peak corresponding to a molecular weight of 8500, which is compatible with the size of known human AA proteins (13). The sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of the retarded protein fraction of E. L. and B. K. were also identical (Chart 1B) with 2 closely migrating protein bands in the molecular weight range of 8000 to 9000. The 2 bands most probably reflected heterogeneity with respect to size in the C-terminal end of both proteins (21).

**DISCUSSION**

The results of the present study showed clearly that AA comprised a principal fibril component in 2 cases of systemic amyloidosis associated with nonmalignant diseases (6, 18, 21).
amyloidosis associated with cancer, namely renal cell carcinoma and mesothelioma, although the histological diagnosis of the latter tumor was not completely verified. This has been suggested previously by us on the basis of immunological studies, which revealed a close antigenic relationship between AA and the same 2 cases of cancer-associated amyloid (17), but a definite proof for this association has not been reported before (9). AA is thus distributed widely in systemic amyloidosis including cases associated with cancer. The other principal type of protein that makes up systemic amyloid fibrils, namely AL protein, has been found mostly in cases of idiopathic amyloidosis (which can be regarded as a form of immunocyte dyscrasia) (9, 13) and those associated with the monoclonal gammopathies, myelomatoses and macroglobulinemias. Even among such cases, AA-type amyloid has indeed been found (17). However, the 2 cases reported here are certainly not enough to permit the conclusion that cancer-associated systemic amyloidosis is always of the AA type. It can be mentioned that serum M-components are found frequently in patients with nonreticular tumors (33), and Bence-Jones proteins, potential precursors of AL-type amyloid, have been found in the urine of patients with renal cell carcinoma (7). More studies are needed, therefore, to see if also the immunoglobulin (AL) type of systemic amyloid fibrils can be associated with these malignant diseases. Moreover, it cannot be excluded that proteins other than AA and AL may act at amyloid precursors in such cases, although these 2 types of protein are the only ones so far reported to be the major component of nonfamilial systemic amyloid fibrils (13).

In both cases, amyloid was also found to be located in the tumors, but the deposits were sparse and limited to the vascular framework of the renal cell carcinomas and to the capillary vessels of the mesothelioma. The vascular distribution of amyloid in the tumors suggests that it is a part of the systemic amyloidosis rather than an actual product of the tumors. SAA, an acute-phase protein and the possible serum precursor of AA-type amyloid, has recently been found to be produced by the liver (25) after stimulation by a factor from activated macrophages (26). Tumor cells have been shown to be effective activators of macrophages (22), and one may suggest, therefore, that the tumor, like inflammatory processes, initiates amyloid formation by such a mechanism. Another possible mechanism may be a defective enzymatic breakdown of SAA, somehow associated with the cancer, leaving AA-like fragments available for amyloid formation, instead of completely split peptides, which could be more readily removed from the body. In vitro studies using treatment of SAA with enzymes of monocytic origin from patients with and without AA-type amyloidosis indicated an incomplete splitting of SAA in those patients who developed amyloidosis (8).

Inasmuch as renal cell carcinoma is associated relatively frequently with secondary amyloidosis, this tumor may be more capable of stimulating amyloid protein production than are others, which may be attributed to the relatively slow growth of this tumor with a consequent long-term stimulative effect (29). In contrast, only one single case of amyloidosis associated with mesothelioma has, to our knowledge, been reported before (4). However, the growth rate of this tumor may vary considerably (1), and in our case this tumor, which was not detected before autopsy, might have been present for a long time. The degree of dissemination of neoplastic tissue seems to be less important for amyloid formation, since many cases of reactive amyloidosis associated with nonmetastatic tumors have been reported (29), and this was also the case with our 2 patients. It is interesting, however, that high serum concentrations of SAA have been found to be associated with the dissemination of cancer (24). However, factors other than high serum levels of SAA are indeed needed for the formation of reactive systemic amyloidosis (8, 13). The mechanisms by which SAA is converted to AA and deposited as amyloid fibrils in the tissues are far from being understood.

**ACKNOWLEDGMENTS**

The authors wish to thank Drs. Frydias Langmark and Helge Stalsberg for providing amyloidotic tissues and for help with the histological diagnoses, Anita Skoge for typing the manuscript, and Kirsti Renne for technical assistance.

**REFERENCES**


21. Neyzer, K., Sletten, K., Husby, G., and Natvig, J. B. An unusually large (83 amino acid residues) amyloid fibril protein AA from a patient with Walden-


Amyloid A in Systemic Amyloidosis Associated with Cancer

Gunnar Husby, Gudmund Marhaug and Knut Sletten


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/4/1600

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.