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Papers

Light chain amyloidosis of the urinary bladder. A site restricted deposition of an externally produced immunoglobulin

A Livneh, S Shtrasburg, B M Martin, J Baniel, R Gal, M Pras

Abstract

***Aims*—To identify the amyloid protein in a patient with amyloidosis localised to the urinary bladder, and to see whether subtyping of the protein by sequence analysis increases the understanding of the selection of the urinary bladder as the site of amyloid deposition.**

***Methods*—A patient with gross haematuria and a congophilic mass in his urinary bladder was evaluated further. Characterisation of the amyloid protein was performed using conventional histological and immunohistochemical methods. Determination of the N-terminal amino acid sequence of the amyloid protein was performed using protein sequencers.**

***Results*—The patient's history, physical examination, and laboratory evaluation excluded the involvement of other organs, justifying a diagnosis of amyloidosis localised to the urinary bladder. Histological and immunological studies showed that the amyloid protein deposited in the urinary bladder of the patient was probably of the amyloid light chain type. No plasma cells or lymphocytes were seen in sections of the urinary bladder and lower ureter adjacent to the amyloid deposits. Molecular analysis showed the sequence NFMLTQPHSISGSPG, which assigned the amyloid protein to either the $V\lambda_I$ or the $V\lambda_{VI}$ immunoglobulin (Ig) light chain families.**

***Conclusions*—The findings suggest that the amyloid protein in this patient originated outside the urinary bladder. The heterogeneity of the Ig proteins in known cases of amyloidosis of the lower urinary tract suggests that the amino acid residues, which determine the Vλ subtyping, have no major role in restricting the deposited protein to the urinary bladder.**

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Keywords: primary amyloidosis; urinary bladder; λ light chain; amino acid sequence

Heller Institute of Medical Research, Sheba Medical Center, Tel-Hashomer 52621, Israel
A Livneh
S Shtrasburg
M Pras

Clinical Neurosciences Branch, National Institute of Mental Health, National Institute of Health, Bethesda, MD, 20892-4405 USA
B M Martin

Department of Urology, Belinson Campus, Rabin Medical Center, Petach-Tiqva, 49100 Israel
J Baniel

Department of Pathology, Golda Campus, Rabin Medical Center, Petach-Tiqva, 49372 Israel
R Gal

Correspondence to:
Professor Livneh
alivneh@post.tau.ac.il

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Amyloid light chain (AL) amyloidosis is characterised by tissue infiltration with a fibrillar protein, consisting of immunoglobulin (Ig) light chain fragments.[1] Using Congo red staining and polarised light microscopy, this proteinaceous material displays a typical green birefringence, which defines it as amyloid.[2] AL amyloidosis usually involves multiple organs. Localised AL amyloidosis is an uncommon type of AL amyloidosis in which, instead of widespread organ involvement, amyloid deposits are limited exclusively to one organ.[3–5] Causes for selection of the specific organ are not known. Local secretion of the amyloidogenic Ig versus distant (usually bone marrow) production of an Ig with an increased affinity for the affected tissue are the current considered alternatives. Apart from being scientifically intriguing, the site of origin of the Ig causing localised AL amyloidosis has important diagnostic and therapeutic implications.

Localised amyloidosis of the lower urinary tract is one of the localised AL amyloidoses. It is an uncommon disease, which has a typical presentation, with gross haematuria and findings mimicking neoplastic tumour of the urinary tract.[6 7] Reports of the chemical characterisation of the amyloid protein are sparse. In the small number of cases where characterisation was undertaken, the Ig deposits were almost always of the λ light chain type.[8 9] Subtyping of the λ chain by immunological and molecular analyses, which may increase our understanding of the preferential deposition in the urinary tract, were only rarely performed.[10–12] In the following report, we present a patient with AL amyloidosis, localised to the urinary bladder, in whom characterisation of the amyloid protein was aided by sequence analysis. The findings in our study may shed light on the question of selection of the urinary bladder as a site for amyloid deposition.

Patient and methods

CASE HISTORY

A 57 year old Jewish man of Iraqi extraction, with an insignificant past medical history, presented in June 1996 to the urology department with macrohaematuria of several days duration. Cystoscopy, abdominal ultrasound, and intravenous pyelography revealed a 2 × 2 cm mass in the left bladder wall, associated with stenosis of the distal part of the ureter,

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hydronephrosis, and hydroureter on the same side. Physical examination at that time was unremarkable, and routine laboratory studies were within normal limits.

In September 1996, transurethral resection (TUR) of the bladder "tumour" and stenting were done. However, following the pathology results, the patient was operated on again and a resection of the distal ureter with bladder cuff and reimplantation were performed. The histology of the excised tissue showed heavy amorphous eosinophilic deposition. Congo red staining was typical for amyloid. Amyloid deposits were found in the ureter also. Immunohistochemical studies, using specific commercial antibodies, (anti-κ and anti-λ: BioGenex, San Ramon, USA; anti-β_2M: Zymed, San-Francisco, USA; anti-AA and anti-prealbumin: Dako, Copenhagen, Denmark), were negative for amyloid A (AA), AL, amyloid transthyretin (ATTR), and amyloid β_2 microglobulin (Aβ_2M). AA was also excluded using the highly sensitive and specific Shtrasburg method.[13 14] No plasma cells or lymphocytic infiltrates were seen in bladder or ureter sections.

Although standard methods failed to classify the amyloid protein in our patient, the results were interpreted as suggesting AL amyloidosis, not identified by the commercial anti-Ig light chain antibodies because of their inability to detect some of the immensely variable proteins found in AL amyloidosis. Further studies were aimed at the detection of other organ involvement. Rectal biopsy, bone marrow examination, echocardiography, abdominal ultrasound, electrophoresis of serum proteins, kidney and liver function tests, serum electrolytes, albumin, globulin, calcium, phosphorous, iron, T3, T4, thyroid stimulating hormone, prothrombin time, haemoglobin, mean corpuscular volume, haematocrit, and urine Bence Jones proteins were all negative or within normal limits.

About two years after the initial lesion was excised, a recurrence of the disease, which now affected much larger portions of the urinary bladder, occurred. TUR of the amyloid deposits was performed again. There was no evidence of systemic amyloidosis. For classification purposes, the amyloid protein was extracted from samples of the urinary bladder tissue obtained at presentation and subjected to sequence analysis as detailed below.

EXTRACTION AND PURIFICATION OF AMYLOID PROTEINS

About 1 g of formalin fixed, paraffin wax embedded bladder tissue was dewaxed, homogenised, and washed, as described previously.[13 14] The resulting sediment was treated with 8M guanidine HCl in 1M acetic acid, dialysed, lyophilised, and dissolved in 98% formic acid. After evaporation of formic acid, the samples were suspended in loading buffer and subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).[14 15] The protein bands were detected by staining with Coomassie brilliant blue and transferred from an analogous intact gel to an immobilon P membrane (Millipore, Bedford, Massachussetts, USA), using a Bio-rad transblot cell, and CAPS buffer. The transfer of the proteins was confirmed by amido black staining.[16]

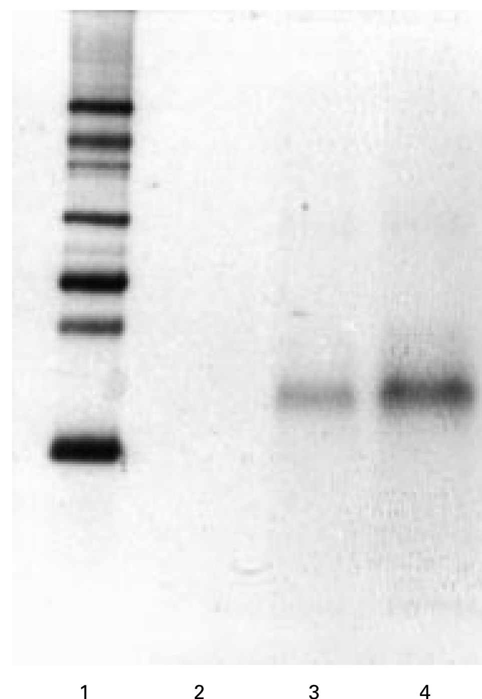

Figure 1 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (17% gel) of extracts of the urinary bladder. The samples were obtained from our patient's lesion and from histologically normal looking urinary bladder control. Lane 1, molecular weight (MW) markers (Dalton mark VII-L; Sigma, St Louis, USA). The bands from top to base indicate a MW of 66, 45, 36, 29, 20.1, and 14.2 kDa. Lane 2, normal urinary bladder tissue subjected to the same extraction and purification protocol. Lane 3, amyloid containing urinary bladder tissue, showing a single band protein with MW of about 17.5 kDa. Lane 4, similar to lane 3—another preparation from the same patient.

N-TERMINAL SEQUENCE DETERMINATION

The band of interest was excised from the immobilon P membrane and its N-terminus was sequenced, using both an ABI 476A sequencer and a Beckman LF3000G protein sequencer. Both sequencers were equipped with online PTH amino acid analysers. Standard sequencing protocols were used, according to the manufacturer's programs. Upon completion of the sequence run, the resulting N-terminal amino acid sequence was searched against GENBANK using NCBI's BLAST search tool to identify the protein.[17]

Results

SDS-PAGE analysis demonstrated a single protein band of 17.5 kDa (fig 1). Partial N-terminal sequence analysis of 15 residues revealed the sequence to be NFMLTQPH-SISGSPG. By homology with previously published Ig sequences, the sequence of the amyloid protein appeared to best fit that of an Ig of the $V\lambda_I$ or $V\lambda_{VI}$ subgroups (13 of 15 identities in each). This finding also confirmed that the amyloidosis of the urinary bladder in our patient is of the AL type.

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Table 1 Features of amyloid light chain amyloidosis of the lower urinary tract of patients in whom the amyloid protein was sequenced

Patient	*Age (years)*	*Site*	*Clinical presentation*	*Treatment*	*Duration of follow up (years)*	*Result*	*Type*	*Subtype*	*Ref*
1	29	Urethra	Haematuria + dribbling	Excision	2	Well	Nodular	$V\lambda_{VI}$	10
2	65	Ureter	Haematuria	Excision	2	Well	Nodular	$V\lambda_{II}$	11
3	50	Ureter	Haematuria + flank pain	Excision	?	?	Nodular	$V\lambda_{III}$	11
4	68	Ureter	Obstruction	Resection	?	?	Nodular	$V\lambda_{II}$	12
5	57	Bladder	Haematuria	Excision	2	Recurrence	Initially nodular. Eventually diffuse	$V\lambda_{I}$ or $V\lambda_{VI}$	Our report

Discussion

We present a patient who, following evaluation for haematuria, was found to suffer from amyloidosis of the urinary bladder. Further studies and the patient's history excluded involvement of other organs, justifying a diagnosis of localised amyloidosis of the urinary bladder. N-terminal amino acid sequence analysis of the isolated amyloid protein revealed that it was homologous to an Ig light chain of either the $V\lambda_{I}$ or the $V\lambda_{VI}$ subgroups, implying that the patient's amyloidosis is of the AL type.

Localised AL amyloidosis of the lower urinary tract (bladder, distal ureter, and urethra) is an uncommon disease, with only around 100 patients reported over the past 30 years (Medline search). The mechanism restricting amyloid deposition to the urinary bladder in almost all patients is unknown, but it may be one of the following: retention in the urinary bladder of a locally produced Ig and/or deposition of a remotely produced Ig in the urinary bladder. Therefore the site of the Ig production is an important clue for the enigma of urinary bladder selection. It is also of therapeutic importance, because in addition to the local surgical management, adjuvant chemotherapy or bone marrow transplantation should be considered for patients in whom the Ig is produced outside the urinary bladder. For amyloidosis restricted to the urinary bladder, such a treatment protocol would be new. To date, the only systemic treatment tried in this disease is colchicine.[18]

Clues to the source of the deposited protein may be derived from the shape of the amyloid deposition.[11] Whereas a nodular form, limited to one or several loci in the urinary bladder, suggests in situ Ig production by an abnormal clone of tissue plasmacytes,[10] a diffuse form, scattered throughout the involved tissue, suggests distant Ig production. The presence of Ig secreting cells close to the amyloid fibrils may lend further support for local generation of the amyloid protein.[8] Demonstration of reactivity of the Ig (which is the variable region of an antibody with antigen binding capacity) with urinary tract antigens is in concert with a distant origin of the deposited Ig. In our patient, the diffuse pattern of amyloid deposition, developed late in the course of the disease, and the absence of lymphocytes or plasma cells in the vicinity of the amyloid deposits, favours the option of distant production of a urinary bladder reactive Ig. The large quantity of amyloid in the urinary bladder tissue of our patient, in sharp contrast to the absence of Ig producing cells around and within the amyloid sediments, is another argument against local Ig production by subtle B cells, not revealed by the standard histological methods.

The sequence analysis of the amyloid protein in our case was undertaken to confirm the Ig origin of the amyloid, and to see whether the structure of the protein may shed light on the issue of urinary bladder selection. In the small number of cases of urinary bladder amyloidosis where the amyloid protein was characterised, it was a fragment of an Ig λ light chain. Only rarely did the authors go one step further to sequence and define the Ig subgroup. Table 1 shows that three different subtypes of λ chain fragments have been associated with localised amyloidosis of the urinary tract, $V\lambda_{II}$, $V\lambda_{III}$, and $V\lambda_{VI}$. The Ig found in our patient was of the $V\lambda_{I}$ or $V\lambda_{VI}$ subgroups. The absence of a urinary tract specific Ig suggests that the selection of the urinary bladder is not determined by primary structural elements, which assign the deposited protein to a certain Vλ group.

Recently, Asl *et al* described a patient with ureter amyloidoma consisting of Ig light chain of the $V\lambda_{II}$ type.[12] Transcripts, obtained from infiltrates of plasma cells within and around the amyloid fibrils, contained Ig light chain sequences identical to the sequence of the amyloid protein. These findings definitely indicate that in that particular patient, and probably in several other patients, lower urinary tract amyloid is produced locally. However, our case and most of the other published cases are characterised by the absence or paucity of local Ig producing cells; therefore, a remote Ig synthesis site for the deposited protein remains a valid possibility. Adding molecular analysis to the regular immunohistochemical methods, Setoguchi *et al* found evidence for monoclonal Ig synthesis in 13 of 16 patients with localised amyloidosis,[19] one of whom had amyloidosis of the urinary bladder. All biopsy specimens had lymphocytes or plasma cells at the site of amyloid deposition. A distant origin of a localised amyloid protein was therefore possible for three patients even when B cells were present around the amyloid infiltrate.

In conclusion, we believe that the amyloid protein in our patient probably originated outside of the urinary bladder. This is suggested by the recurrence of the disease, despite local excision, the diffuse involvement of the urinary bladder at the relapse of the disease, and the absence of plasma or B cells in the involved tissue. Sequence analysis suggests that the urinary bladder affinity of this remotely produced Ig is not related to the residues that determine its specific Vλ subtype. The source of the Ig should be determined in any patient with localised amyloidosis because it may vary

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(be either local or distant) and thereby affect individual treatment protocols.

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