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# Rapid Communication

## Light Chain Deposition Disease Derived from the $\kappa_I$ Light Chain Subgroup

### Biochemical Characterization

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***The authors biochemically analyzed the nonamyloidotic light chain deposits, the first studied in this way, from a patient with systemic κ light chain deposition disease (LCDD). The light chain deposits from myocardium were extracted in 6 M guanidine-HCl under reducing conditions, partially purified by column chromatography, and analyzed by immunoblotting and amino-terminal sequencing. The extracted material contained four main bands reactive with anti-κ antibody: intact κ light chain (MW, 28 kd), under reducing conditions, and 3 fragments (MW, 20, 16, and 15 kd). As revealed by the aminoterminal sequencing performed on three of the four bands, the intact light chain molecule and two fragments belong to the $\kappa_I$ subgroup. Thus, similar to light chain amyloid (AL), the deposits in LCDD are derived from both intact light chain and fragments. Unlike in AL, amyloid P component was not detected in the deposits of this patient or those examined previously. The differences demonstrated thus far between AL and LCDD are the lack of fibrils and amyloid P component in LCDD, suggesting that local tissue factors may be responsible for different processing of the light chain deposits in LCDD. (Am J Pathol 1989, 134:749–754)***

The plasmacytic dyscrasias are characterized by an abnormal proliferation of monoclonal plasma cells and the excess production of monoclonal immunoglobulin that may deposit and accumulate in tissues with impairment of organ structure and function. Ultrastructurally, the deposits may be fibrillar, as in amyloid derived from light chain (AL), or nonfibrillar and granular, as in light chain deposition disease (LCDD). In the case of AL, the deposits exhibit congophilia and green birefringence when examined under polarized light, whereas in LCDD, the deposits are noncongophilic and nonbirefringent. The common diagnostic feature of the deposits in AL and LCDD is the detection of a single light chain isotype.[1,2]

LCDD was first noted in renal biopsies in 1973 and fully described in 1976.[3,4] Since then, approximately 100 cases of LCDD associated with lymphoplasmacytic disorders have been reported.[5,6] The incidence in myeloma and plasma cell dyscrasias is unknown. The diagnostic feature is the diffuse staining of basement membranes for a single light chain isotype, either κ or λ.[1–6] Ultrastructurally there are usually corresponding clusters of punctate, granular, electron-dense deposits in basement membranes. Although most patients have renal symptoms and the diagnosis is often first established by renal biopsy, tissues examined at autopsy reveal that the deposits are systemic and other clinical manifestations such as hepatic and cardiac symptoms may be prominent.[2,4–6] The diagnosis of LCDD is usually supported by the detection of a monoclonal light chain of the same isotype in serum and/or urine, detection of a monoclonal proliferation of plasma cells bearing the same light chain isotype in bone marrow examined by immunopathologic methods, or by

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the demonstration of a corresponding excess production of monoclonal light chains in bone marrow cell cultures.[1,5]

Although biochemical analysis of AL fibril protein has been done in a number of cases, no such studies have been reported in LCDD for comparison. We describe here the immunohistochemical and biochemical characterization of the nonamyloidotic light chain deposits from cardiac muscle of a patient who died from systemic LCDD.

## Materials and Methods

The tissue for extraction of the light chain deposits was obtained at autopsy from a 23-year-old white man (McM) with myeloma. The diagnosis of LCDD was established by immunofluorescence microscopic examination of biopsies from myocardium, rectum, and skin, which demonstrated typical deposits of $\kappa$ light chain in basement membranes, and by electron microscopy, which showed characteristic clusters of granular deposits (Figure 1A,B). The main findings at autopsy were cardiomegaly (1300 g), hepatomegaly (3000 g), and splenomegaly (250 g). Routine immunofluorescence microscopic examination[2] revealed $\kappa$ light chain deposits in systemic basement membranes of spleen, heart, lung, kidney, liver, pancreas, prostate, thyroid, testes, intestines, skin, and choroid plexus. Stains for $\lambda$ light chain, heavy chains ($\gamma$, $\alpha$, $\mu$), $C_3$, C1q, and amyloid P component were negative. Sections from all organs stained with Congo red revealed no evidence of amyloid.

### Extraction of the Light Chain Deposits

The light chain deposits were extracted as described with slight modification.[7] Briefly, 10 g of frozen myocardium, stored at −70 C, was homogenized, repeatedly washed in phosphate-buffered saline (PBS) (10 × volume), and centrifuged. The procedure was continued until the optical density of the washings measured at 280 nm was 0.270. All steps were carried out at 4 C and to prevent proteolysis, 1 mM phenylmethylsulfonylfluoride (Sigma Chemical Co., St. Louis, MO) was added to the washing buffer. Extraction was monitored by examination of supernates and pellets using immunoblotting, and smears of the pellets by direct immunofluorescence. An aliquot of the pellet (1.2 g wet weight) was extracted in 6 M guanidine-HCl in 0.050 M TRIS buffer, pH 10.2, with 0.17 M DTT for 48 hours at room temperature with slow stirring. The guanidine extract was centrifuged at 100,000*g* for 1 hour at 4 C and the supernate was fractionated by Sephadex G-100 (Pharmacia, Piscataway, NJ) column chromatography (150 × 2.5 cm) in 5 M guanidine HCl in 1 M acetic acid. The fractions were dialyzed extensively against distilled water in a membrane tubing with a cutoff of 3.5 kd, lyophilized, and subjected to further analysis with SDS-PAGE, immunoblotting, and amino-terminal microsequencing.

### Immunoblotting

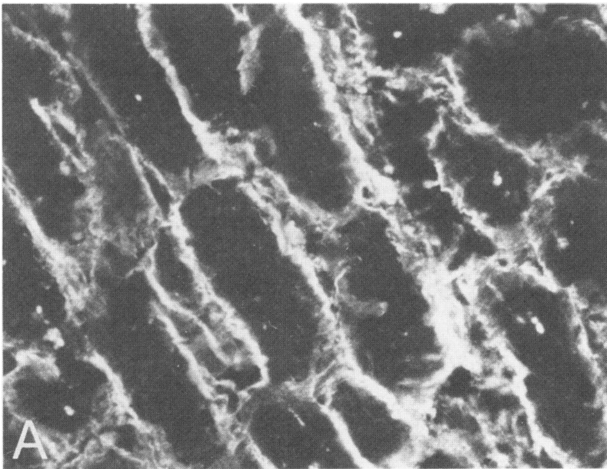
Immunoreactivity of the samples was analyzed after SDS-PAGE electrotransfer to Millipore Immobilon polyvinylidene difluoride (PVDF) transfer membrane as described.[7] The primary antisera used in the study were anti-human $\kappa$ and anti-$\lambda$ light chains (1:1000, 1:2000, 1:4000, 1:8000; DAKO, TAGO, and Amersham, affinity purified). After extensive washing in buffer, bound antibody was detected with a biotin-streptavidin system (Amersham, Arlington Heights, IL). Peroxidase activity was visualized with 3.3′ diaminobenzidine (Sigma).

### Aminoterminal Microsequencing

Samples for amino-terminal automated microsequencing were isolated by electroblotting.[8] Briefly, immediately after SDS-PAGE, electroblotting onto PVDF transfer membrane was performed in a Bio-Rad mini trans-blot electrophoretic transfer cell at room temperature with cooling for 1.5 hour (50 V) using TRIS-glycine buffer, pH 8.3, in 20% methanol with 0.001% SDS. Aliquots of the material for electroblotting and marker proteins were applied into several wells of one gel. The electroblotted bands were detected using Coomassie brilliant blue R-250; their immunoreactivity was confirmed on the adjacent lanes by immunoblotting. The protein bands reactive with anti-$\kappa$ light chain antibody were cut out from the membrane and sequenced in a 470A protein sequencer (Applied Biosystems [ABS], Foster City, CA) and the PTH amino acids were identified using an ABS on-line 120A PTH analyzer and the standard ABS program.

## Results

Immunofluorescence staining of the smears from pellets performed after multiple washings in PBS confirmed the presence of the $\kappa$ light chain bound to myocardial cell fragments, and absence of $\lambda$ light chain and amyloid P component. Immunoblotting examination of the myocardial pellets revealed both intact and fragments of $\kappa$ light chain but only trace amounts of $\lambda$ light chain, a likely contaminant from the serum. The washings contained both $\kappa$ and $\lambda$ light chains; both intact molecules and fragments



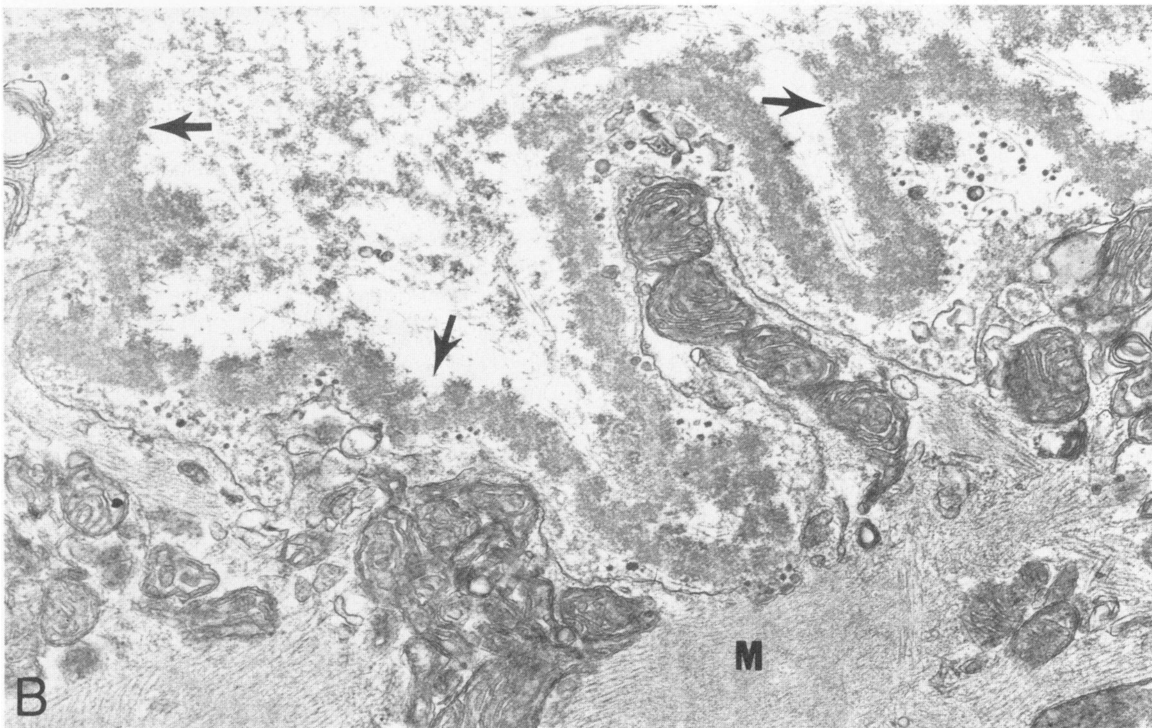




Figure 1. *Myocardium.* A: *Frozen section stained with fluorescein conjugated anti-κ antibody shows strong staining of membranes around myocardial cells (×300).* B: *Electron micrograph demonstrates typical clusters of granular and punctate electron dense deposits (arrows) in membranes around muscle cells (M), characteristic of light chain deposits lacking the fibrillar appearance of amyloid (×25,000).*

of κ light chain were detected, whereas only intact λ light chain, which diminished with successive washings, was demonstrable.

The SDS-PAGE analysis of the pellet after the last washing in PBS revealed high molecular weight aggregates (particularly abundant under nonreducing conditions), and smaller proteins ranging in size between 100 and 14 kd under reducing conditions. Under reduced conditions and when stained for κ light chain, the blots showed four distinct bands with approximate molecular weights of 28, 20, 16, and 15 kd; the largest corresponded to an intact light chain and the others to fragments of the light chain. A slightly slower electrophoretic mobility of the intact light chain giving the estimated molecular weight of approximately 28 kd, appears to be due to reducing conditions, because under nonreducing conditions the corresponding band has a molecular weight of approximately 25 kd. Although it is possible that carbohydrate groups could be responsible for the observed increased molecular weight, this was not tested. Goni et al. have described an unglycoslyated light chain that has a similar molecular weight (28 kd) under reducing conditions.[9]

The protein extracted in guanindine under reducing conditions was fractionated on a Sephadex G-100 column as described previously.[7] This resulted in a relatively

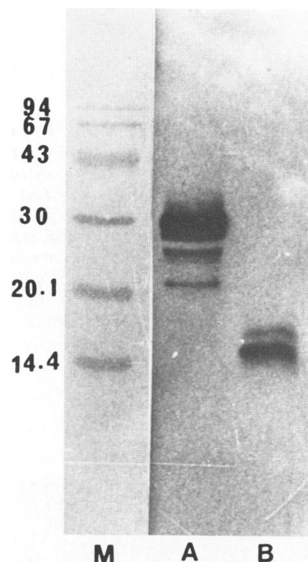



Figure 2. *Immunoreactivity of the bands selected for the aminoterminal sequencing. Lane M: Marker proteins from top to bottom: phosphorylase B 94 kd, bovine serum albumin 67 kd, ovalbumin 43 kd, carbonic anhydrase 30 kd, soybean trypsin inhibitor 20.1 kd, and α-lacto-albumin 14.4 kd. Lane A: Intact κ light chain, (molecular weight of approximately 28 kd (fraction 1). Lane B: Two fragments of the light chain, MW approximately 16 and 15 kd (fraction 3). Reduced conditions, all lanes.*

minor further purification of the material, because no separation into distinct peaks was achieved (not shown). The eluate consisted of a void peak, which was not studied further, followed by three fractions. As revealed by SDS-PAGE, only the last fraction (#3) was relatively uniform in that it contained only 3 bands, (MW, 14 to 16 kd), two of which are clearly reactive with anti-κ antibody (Figure 2, lane B). Fractions 1 and 2 contained a number of bands ranging in size from approximately 30 to 20 kd. Thus, in addition to an apparently intact κ light chain (MW, approximately 28 kd) in fraction 1 and a 20 kd fragment in fraction 2, there were several other bands. Among these were bands that were clearly nonreactive with anti-κ light chain antibody, as well as a number of relatively thin bands reactive with anti-κ light chain antibody (Figure 2, lane A).

Even though the extracted material was not purified to homogeneity, it was possible to perform the aminoterminal sequencing on selected distinct bands reactive with anti-κ antibody. Three bands reactive with anti-κ light chain antibody were examined by the aminoterminal sequencing: these were 28 kd from fraction 1 (Figure 2, lane A) and 16 and 15 kd from fraction 3 (Figure 2, lane B). All three amino-terminals belong to the $\kappa_1$ light chain subgroup, the largest protein representing most likely an intact light chain and the two smaller ones fragments of the complete variable and part of the constant region (Figure 3).

## Discussion

The light microscopic and immunohistochemical studies in this patient with myeloma demonstrate the diagnostic features of LCDD, ie, monotypic nonfibrillar and noncongophilic κ light chain deposits in the systemic basement membranes of many organs including heart, kidney, lung, liver, spleen, thyroid, prostate, pancreas, small intestine, rectum, skin, and choroid plexus. The deposits in all organs lack the amyloid P component, a glycoprotein that has been found in all amyloid deposits, which confirms our previous findings.[2]

Our biochemical studies reveal that, as in light chain amyloid, the deposits in LCDD are composed of both intact light chain and fragments thereof. The light chain deposits in this patient, the only one analyzed thus far, are derived from the $\kappa_1$ subgroup. Only a few amino acid sequence studies of AL proteins are published;[10-14] of the ten amyloid κ proteins,[14] eight are of the same $\kappa_I$ subgroup as the nonamyloid protein deposits in the present case of LCDD. The reported molecular weights of the purified amyloid proteins derived from the κ light chain range between 7.5 and 23 kd (7.5; 18.3; 22 and 17; 23 and 14.5; 12; 22; 15; 13; 14; 17; 13.8).[10-14] Thus, although there are no consistent differences in the range of sizes of light chain protein between AL and LCDD, the size distributions do differ, at least based on the comparison with this single patient. It appears that in the nonamyloid light chain deposits of LCDD, the predominant component is the intact light chain, whereas in AL, fragments, many of which are relatively short, prevail.[10-14] Fragments of the light chain, similar in size to those in our patient, also were reported previously in the urinary Bence Jones protein in another patient with multiple myeloma and κLCDD.[15] In a single recent report of extracted protein tissue deposits in LCDD only one fragment (MW, 17 kd) and no intact light chain was identified.[16]

The reason why fibrillogenesis occurs in the light chain deposits of AL, but not LCDD, is not known; however, unusual aminoacid sequences and substitutions were reported in several amyloid proteins.[13] A comparison of amyloid and nonamyloid myeloma $\kappa_I$ proteins suggested a chemical difference between the groups, ie, substitutions of hydrophilic framework residues by hydrophobic amino acids in amyloid proteins.[13] These substitutions may provide nucleation sites for self aggregation and fibril formation. Although presently not available, further comparison with the complete amino acid sequence of the nonamyloid deposits in LCDD might also demonstrate differences that could provide additional insight into AL fibril formation.

Of special interest are those individuals in whom both types of deposits, ie, amyloid and nonamyloid, of the

| | | | | | 5 | | | | | 10 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$McM_{28}$** | **ASP** | **ILE** | **GLN** | **MET** | **THR** | **GLN** | **SER** | **PRO** | **SER** | **THR** | **LEU** | **SER** | **ALA** | **SER** |
| **$McM_{16}$** | - | - | - | - | - | - | - | - | - | - | - | | | |
| **$McM_{15}$** | - | - | - | - | - | - | - | - | - | - | - | - | | |
| **ROY** | - | - | - | - | - | - | - | - | - | **SER** | - | - | - | - |

**Figure 3.** *The amino terminal sequence of the protein McM: intact light chain* ($_{MW\ 28\ kd}$) and two fragments ($_{MW\ 16\ dk}$) and ($_{MW\ 15\ kd}$) in comparison with protein ROY[12] of the same V region subgroup ($V_{\kappa I}$)–indicates identity.

same light chain isotype coexist.[2,5] Interestingly, the distribution of amyloid and nonamyloid deposits is different, ie, either in different organs, or different sites within the same organ. In such cases, local differences in the degradation and size of the protein (as shown previously in amyloid deposits from different sites[17–19]) may depend on local tissue factors for fibril formation. Amyloid-enhancing factor[20] or amyloid P component, found in AL but not in LCDD,[2] could be important in this regard. Alternatively, the protein structure of the coexistent fibrillar and nonfibrillar deposits may differ and could best be determined by amino acid sequence analysis of the two types of deposits. As shown in the analysis of the $\kappa_I$ amyloid protein BAN, the V region sequences of the light chain may be identical but the J segment sequences differ, indicating the selection of a different J segment DNA fragment.[13] Answers to these questions await further studies.

The reasons why only some patients with gammopathy develop LCDD also are not clear. The distribution of the deposits that outline basement membranes is unique and similar to that seen after the intravenous injection of cationic heterologous proteins in experimental animals.[21] Conceptually, an autologous light chain with excessive amino groups that impart a positive charge could bind to anionic sulphated proteoglycans that are present in basement membranes.[22]

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