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Somatic Mutations of the L12a Gene in V-κ_1 Light Chain Deposition Disease

Potential Effects on Aberrant Protein Conformation and Deposition

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Light chain deposition disease (LCDD) and light chain amyloidosis (AL) are disorders of monoclonal immunoglobulin deposition in which normally soluble serum precursors form insoluble deposits in tissues. A common feature in both is the clonal proliferation of B-cells that produce pathogenic light chains. However, the deposits in LCDD differ from those in AL in that they are ultrastructurally granular rather than fibrillar and do not bind Congo red or colocalize with amyloid P component or apolipoprotein E. The reason(s) for their differences are unknown but are likely multifactorial and related to their protein conformation and their interaction with other molecules and tissue factors in the microenvironment. Knowledge of the primary structure of the light chains in LCDD is very limited. In the present study two new κ_1 light chains from patients with LCDD are described and compared to seven other reported κ-LCDD proteins. The N-terminal amino acid sequences of light chain GLA extracted from the renal biopsy and light chain CHO from myocardial tissue were each identical to the respective light chains isolated from the urines and to the V-region amino acid sequences translated from the cloned cDNAs obtained from bone marrow cells. The germline V-region sequences, determined from the genomic DNA in both and in MCM, a previously reported κ_1 LCDD light chain, were identical and related to the L12a germline gene. The expressed light chains in all three exhibit amino acid substitutions that arise from somatic mutation and result in increased hydrophobicity with the potential for protein destabilization and disordered conformation. ***(Am J Pathol 1999, 155:2009–2017)***

The monoclonal immunoglobulin deposition diseases, which include nonamyloid light chain deposition disease (LCDD) and light chain amyloidosis (AL), have in common monoclonal immunoglobulin synthesis by an expanded clone of B cells leading to the deposition of insoluble light chains in systemic organs with displacement and eventual destruction of parenchymal cells and organ dysfunction.[1] However, LCDD and AL differ in several ways. In the case of AL the deposits are congophilic, have a fibrillar ultrastructure, are more frequently derived from λ than κ light chain, have a patchy distribution within and among systemic organs, and colocalize with amyloid P component, apolipoprotein-E (apo-E), and glycosaminoglycans (GAGs).[2,3] In contrast, the deposits in LCDD are noncongophilic, have a granular rather than fibrillar ultrastructure, and are more frequently κ than λ. In cases completely examined at autopsy, deposits are uniformly distributed in all the basement membranes of systemic organs[4,5] and do not associate with amyloid P component or apo-E.[2] These differences are of fundamental importance in determining the mechanisms of protein deposition in tissues and the process of fibrillogenesis possibly relevant to other common types of amyloidosis, such as Alzheimer's disease. However, the biophysical basis for their differences is poorly understood.

Knowledge of the primary structure of the protein deposits in LCDD is very limited; in only nine cases of LCDD has the complete light chain variable region (V-region) sequence been published.[6–13] Thus, a comparison of the nonfibrillar and fibrillar forms of deposits in LCDD and AL that could identify differences in their primary structures and might relate to their dissimilar properties is ham-

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pered by the apparent scarcity of cases of LCDD to study and the unavailability of large amounts of tissues from postmortem examinations for biochemical analysis. This limitation is now partially overcome by microextraction methods to isolate and obtain the amino-terminal sequence of light chain deposits from milligram amounts of diagnostic biopsy tissues,[14,15] as well as the application of molecular techniques to obtain the light chain V-region amino acid sequence translated from cloned cDNA of bone marrow cells. These methods, applied to more readily available biopsy tissues, allow the opportunity to build a primary structure data base for comparison with nonpathogenic Bence-Jones light chains as well as those in AL disease, with the goal of elucidating the mechanism(s) of tissue deposition and fibrillogenesis.

In a previous case of LCDD, we reported the biochemical data of a V-region κ_1 (V-κ_1) nonamyloidotic immunoglobulin light chain, MCM, obtained by extraction of deposits from myocardial tissue in which five unique amino acid substitutions were identified.[11] We now report two new κ_1 LCDD proteins: GLA and CHO. In both we determined the N-terminal amino acid sequences of the light chains isolated from the tissues and urines, the complete V-region light chain amino acid sequences deduced from the cloned cDNAs, and the nucleotide germline sequences from genomic DNA. We also determined the germline sequences from genomic DNA of MCM. We conclude that the amino acid substitutions identified in GLA and CHO, as well as in MCM, are due to somatic mutations and contribute to protein instability, aggregation, and the deposition of the light chains in the tissues.

Materials and Methods

Patients

The diagnosis of LCDD was made in two patients who presented with renal disease and whose renal biopsy tissues showed monotypic κ light chain deposits.

A 61-year-old caucasian female (GLA) presented with increasing renal functional impairment. Physical examination revealed no edema or other abnormalities. The serum creatinine rose from 0.8 to 2.6 mg/dl over an 8-month period and to 3.7 mg/dl at the ninth month, the time of admission and renal biopsy. The urine showed microhematuria and a urine protein excretion of 0.16–0.37 g/24 hours. The serum cholesterol level was 270 mg/dl, C3/C4 was 129/40 mg/dl (normal), and hematocrit was 32%. A diagnosis of κ LCDD was made by renal biopsy. Immunoelectrophoresis revealed no monoclonal protein in the serum and a mixture of albumin and monoclonal Bence-Jones κ light chain, estimated at 30 mg/dl, in 100-fold concentrated urine. A bone marrow biopsy showed features of a B-lymphocyte neoplasm. Despite chemotherapy with Melphalan and Prednisone, irreversible renal failure developed, requiring maintenance hemodialysis.

The second patient, a 64-year-old Asian female (CHO), was admitted to the hospital for complaints of fatigue, anorexia, back pain, and acute renal failure that developed after abdominal computed tomography (CT) with radio contrast. She was known to have normal renal function, with a serum creatinine of 0.9 mg/dl, 2 years before. After CT studies the creatinine was 5.7 mg/dl and rose to 9.1 mg/dl 4 days later at the time of admission. The past medical history was unremarkable except for hepatitis B surface antigenemia for many years. Her blood pressure was normal and there was no edema. The significant laboratory findings included urine protein excretion, 1.0 g/24 hour; microhematuria; blood urea nitrogen, 100 mg/dl; serum Ca, 10.4 mg/dl; Phos, 6.7 mg/dl. A diagnosis of κ LCDD was made by renal biopsy. The initial serum and urine immunoelectrophoresis showed no monoclonal Ig, but subsequently Bence-Jones κ light chain was identified in concentrated urine, and lytic lesions were detected in the skull. Bone marrow examination revealed multiple myeloma. Renal failure worsened and expiration occurred 2 months after admission. A postmortem examination was performed.

Pathological and Immunohistological Studies

Paraffin sections of formalin-fixed tissues were stained with hematoxylin and eosin, periodic acid silver methenamine, and Congo red. Standard immunofluorescence microscopy examination of frozen sections was performed on renal biopsy tissues from both patients and on systemic tissues obtained at necropsy from CHO. Sections were incubated with a panel of fluorescein-labeled rabbit polyclonal anti-human Ig chain-specific antibodies (γ, μ, α, κ, and λ), C3, C1q, fibrin and unlabeled rabbit polyclonals anti-amyloid P component (Dako, Carpenteria, CA), and anti-apo-E (Chemicon, Temecula, CA), followed by fluorescein-conjugated swine anti-rabbit Ig (Dako). Immunoperoxidase examinations of formalin-fixed, paraffin-embedded bone marrow biopsy specimens from both patients incubated with polyclonal anti-κ and anti-λ antibodies (Dako) were performed as described.[16]

Electron microscopic studies were carried out on ultrathin sections of glutaraldehyde-fixed epon-embedded renal biopsy tissues and on frozen cardiac tissue stored at −70°C.

Light Chain Isolation from Tissue and Urine Specimens

The GLA residual 1-mm^3 frozen renal biopsy tissue was washed three times in 500 μl of 50 mmol/L phosphate/150 mmol/L NaCl (PBS) (pH 7.2) and centrifuged at 2500 rpm. The tissue pellet was placed in dissociating buffer (50 mmol/L Tris, pH 6.8, 2% sodium dodecyl sulfate, 5% glycerol, 0.1% bromophenol blue), incubated at (80°C) for 2 hours with continuous agitation and centrifuged at 2500 rpm for 5 minutes. The supernatant was boiled for 5 minutes after the addition of dithiothreitol (DTT) to a final concentration of 100 mmol/L.

Light chain deposits were extracted from CHO myocardial tissue stored at −70°C as previously described.[11] Briefly, tissue (5–8 g) was repeatedly homogenized in 10