

Structure of a Monoclonal Kappa Chain of the $V_{\kappa_{IV}}$ Subgroup in the Kidney and Plasma Cells in Light Chain Deposition Disease

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Abstract

That structural abnormalities may be responsible for nonamyloid immunoglobulin (Ig) light chain deposition disease (LCDD) is suggested by previous results of Ig biosynthesis studies, but this hypothesis was not documented at the molecular level. We report on the first complete primary sequence deduced from cDNA analysis of a κ light chain responsible for LCDD associated with an apparently nonsecretory myeloma. Bone marrow myeloma cells contained intracellular κ chains and no heavy chains by immunofluorescence. Kidney biopsy showed typical nonamyloid PAS-positive κ chain deposits. SDS-PAGE analysis of material extracted from a kidney biopsy specimen and of Ig produced by the myeloma cells revealed κ chains of abnormally high apparent molecular mass (30,000). Comparison of the NH_2 -terminal amino acid sequence of the κ chain deposited in the kidney and of the complete sequence of several identical κ cDNA clones from bone marrow cells showed the identity of the tissue deposited and plasma cell κ chain. The κ mRNA had an overall normal structure and corresponded to the $V_{\kappa_{IV}}$ gene rearranged to J_{κ_1} and followed by a normal constant exon of the $Km(3)$ allotype. The variable sequence differed from the $V_{\kappa_{IV}}$ germline gene by nine point mutations, including an Asp \rightarrow Asn substitution at position +70 resulting in a potential N -glycosylation site. In vitro biosynthesis experiments and treatment with N -glycosidase provided evidence for the intracellular glycosylation of the monoclonal κ chain. The peculiar sequence and the glycosylation of a κ chain of the rare $V_{\kappa_{IV}}$ subgroup might be responsible for structural abnormalities leading to tissue deposition. (*J. Clin. Invest.* 1991. 87:2188–2190.) Key words: cDNA sequence • immunoglobulin light chain • nonsecretory myeloma

Introduction

Two diseases featuring tissue deposition of monoclonal immunoglobulin (Ig)-related material, AL-type amyloidosis, and light chain (LC)¹ deposition disease (LCDD), are severe complications of either overtly malignant, mostly multiple my-

eloma, or apparently benign ("primary" forms) plasma cell dyscrasias (1–6). A large body of information has been collected in amyloidosis concerning the nature of the deposited material, serum and urinary Ig, and Ig biosynthesis (1, 4–23). In contrast, data comparing the amino acid sequences of Ig produced by the monoclonal plasma cells with the deposited protein are not yet available in LCDD (5). In a recently published LCDD case, the same 14 NH_2 -terminal V_{κ_1} subgroup amino acid sequence was found in a deposited κ chain and its fragments having relative molecular masses (M_r) of 28,000, 20,000, 16,000, and 15,000 (24). That structural abnormalities of monoclonal Ig may play a pathogenetic role in LCDD is suggested by recurrence of the disease in grafted kidney (25) and by results of in vitro Ig biosynthesis experiments and study of serum monoclonal Ig. In some, but not all patients, the plasma cells produced LC with an abnormal size (either short or apparently large) that were glycosylated and polymerized in vitro and in vivo; when a heavy chain was also present in tissue deposits, it was found to be truncated and there was a correlation between the occurrence of structural abnormalities of Ig chains and their finding in the deposits by immunofluorescence (3, 4, 26–28). In such cases, the abnormal Ig chains were usually not detectable in significant amounts in serum and urine, some patients being affected with so-called nonsecretory myeloma. The occurrence of LCDD and amyloidosis in nonsecretory myeloma is not surprising because plasma cells from several nonsecretory myeloma patients were found to produce and secrete abnormal Ig chains which were undetectable in the serum and urine, probably due to rapid postsecretory degradation and/or tissue deposition (3, 4, 23, 26–29). We herein report on a patient with nonsecretory myeloma and κ LCDD in whom we sequenced the monoclonal LC at the complementary DNA (cDNA) level and showed the deposited LC to have the same NH_2 -terminal sequence. The plasma cell κ chain was N -glycosylated due to a mutation in the variable (V) region, and its apparent M_r was the same as that of the deposited κ chain.

Methods

Patient. A 69-yr-old white woman was referred for rapidly progressive renal failure. Clinical examination was unremarkable. Blood urea nitrogen was 33.1 mmol/liter, creatinine 550 μ mol/liter, glucose 5.5 mmol/liter, potassium 5.2 mmol/liter, calcium 2.1 mmol/liter, phosphorus 1.9 mmol/liter. There was a moderate anemia (hemoglobin 9.6 g/dl, hematocrit 29%) with normal white blood cell and platelet counts. Proteinuria was 0.3 g per day without microscopic hematuria. Total serum protein level was 62 g/liter with 36 g/liter albumin and 6 g/liter gammaglobulins. Search for various autoantibodies and for hepatitis B virus surface antigen and antibody was negative. Total hemolytic complement and C3a, C3c, and C4 levels were normal. Search for monoclonal Ig in the serum and concentrated urine by electrophoretic and immunoelectrophoretic analysis and by a sensitive Western blot technique (30) was repeatedly negative. Pathological and immunofluorescent study of an open renal biopsy showed nodular glomerulosclero-

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1. Abbreviations used in this paper: CDR, complementarity determining region; FR, framework region; LC, light chain; LCDD, light chain deposition disease; PAS, periodic acid-Schiff.

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sis and κ chain deposits in the mesangium, glomerular capillaries, tubular basement membranes, and arterial walls. The deposited material was strongly stained by the periodic acid-Schiff (PAS) reaction. Congo red and thioflavine T staining were negative. Electron microscopic study revealed dense granular deposits without fibrillar organization. Skeletal x-ray survey showed diffuse osteoporosis without lytic lesions. Bone marrow smears contained 10% plasma cells. The bone marrow biopsy pattern was diagnostic of myeloma. Cytoplasmic immunofluorescence of bone marrow cells collected by aspiration before treatment also showed 10% plasma cells which stained for κ light chains and were negative with anti- λ , $-\gamma$, $-\alpha$, $-\mu$, and $-\delta$ conjugates.

Study of the kidney κ chains. The same open biopsy specimen (~50 mg) was used for immunohistochemical, ultrastructural, and biochemical studies. The fragment used for protein study was ground in liquid nitrogen, washed with cold 10 mM PBS, pH 7.4, containing 1 mM PMSF and 5 mM ϵ -aminocaproic acid, and centrifuged at 110,000 g at 4°C for 10 mn. After a second identical washing, the pellet was resuspended in 50 μ l of 1% SDS, 10% glycerol, 1% 2-mercaptoethanol, 62.5 mM Tris/HCl, pH 6.8, buffer (sample buffer). This extract was incubated 10 mn at 70°C and analysed by SDS-12% PAGE (31) followed by electroblotting on nitrocellulose sheets. After saturation with 5% skimmed milk, 0.5% Tween 20, the nitrocellulose was incubated with alkaline phosphatase-conjugated anti- κ antibody (Sigma Chemical Co., St. Louis, MO) and revealed with 0.4 mM tetrazolium nitroblue/bromochloroindolyl phosphate. A similar SDS-PAGE gel containing 10 mM reduced glutathione was transferred onto an Immobilon-P membrane (Millipore, Bedford, MA) according to Matsudaira (32) and stained with Coomassie blue. The band corresponding to κ chains was determined by comparison with an adjacent lane revealed by the anti- κ conjugate, cut out, and used for the determination of the NH₂-terminal amino acid sequence on a gas-liquid solid phase sequencer (470A; Applied Biosystems, Inc., Foster City, CA) (33, 34). The latter analysis was performed by Dr. Denoroy at the Service Central d'Analyse, CNRS (Vernaison, France), without knowledge of the cDNA sequence data.

Nucleic acid studies. Cells from the patient's bone marrow obtained at presentation were washed three times with sterile PBS. Total RNA was prepared by lysis of the cells in 4 M guanidine isothiocyanate followed by centrifugation at 170,000 g during 18 h through a 5.7 M cesium chloride pad (35). Total RNA was analyzed on 1% agarose, 0.7 M formaldehyde gels in comparison with the normal-sized κ mRNA from the Burkitt's lymphoma cell line JI (36), transferred to nylon sheets and hybridized with appropriate DNA probes. The C κ probe was a 2.5-kb Eco RI genomic fragment containing the human C κ exon; the J κ probe was a 1.9-kb Sac I genomic fragment encompassing all five J κ segments; the C λ probe was a 3.5-kb Eco RI-Hind III fragment containing the C λ 2 gene; the C γ probe was a 1.5-kb Pst I fragment containing the hinge, C γ 2 and C γ 3 exons of the C γ 1 gene. A cDNA library was constructed according to standard procedures (35). Briefly, poly(A) mRNA was prepared by affinity chromatography on oligo(dT)-cellulose (Pharmacia Fine Chemicals, Uppsala, Sweden) and used as template for synthesizing single-stranded cDNA by extending oligo(dT) primers with reverse transcriptase (Amersham International, Amersham, UK). Double-stranded cDNA was obtained by adding RNase H and DNA polymerase I, and cloned in the λ gt10 vector using Eco RI adaptors (Amersham International). Recombinant phage was screened with the C κ probe; cDNA clones were digested with the restriction endonucleases Eco RI, Pst I, and Sac I, subcloned into mp18 and mp19 M13 vectors and sequenced by the dideoxy termination method (37) using modified T7 polymerase (United States Biochemicals, Cleveland, OH).

Biosynthetic labeling of bone marrow cells. Biosynthesis experiments were performed on a further bone marrow sample obtained after therapy and containing only 1% tumoral plasma cells. Cells were isolated by sedimentation in 0.8% gelatin (Plasmagel, R. Bellon, Neuilly, France) for 30 mn at 37°C, and internally labeled for 3 h by incorporation of either ³H-leucine (150 μ Ci in 1 ml, sp act 163 Ci/mM) (Amersham International) in leucine-depleted medium supplemented with 2% FCS, or ¹⁴C-glucose (100 μ Ci in 1 ml, sp act 340 mCi/mM) (New

England Nuclear, Boston, MA) in glucose-depleted medium. A control ³H-leucine incorporation experiment was performed with the Burkitt's lymphoma cell line IARC317 producing a normal sized IgA₁ κ . Cells were pelleted and lysed using 0.5% Nonidet-P40 (NP40) in the presence of 2 mM PMSF. Cell extracts and culture supernatants obtained after centrifugation at 15,000 g for 10 mn were precleared with Sepharose coupled to polyclonal human IgG. Labeled Ig were purified by a 2-h incubation at 4°C with a monoclonal anti- κ antibody (clone HP 6023, given by Dr. C. B. Reimer, Atlanta, GA) bound to protein A-Sepharose beads (Pharmacia Fine Chemicals). Immunoglobulins were eluted by boiling for 2 mn in 1% SDS and analysed by 12% SDS-PAGE. Aliquots were submitted to a 30 mn digestion at 37°C by N-glycosidase F (kindly provided by Dr. Y. Karamanos, Limoges, France) in 100 mM Tris, pH 8.6, 20 mM EDTA, 0.5% SDS, 3% NP40, 2 mM PMSF, to be analyzed on the same slab gel. Gels were fixed, stained with Coomassie blue, impregnated with a fluorographic solution (Enhance, New England Nuclear) and autoradiographed for 5 wk.

Results

Large amounts of a normal-sized κ mRNA were found on Northern blots of material extracted from bone marrow cells (Fig. 1). Faint signals were obtained with the C γ and C λ probes and probably corresponded to a small amount of polyclonal lymphoid cells in the first bone marrow sample (not shown). Out of 2 \times 10⁵ cDNA clones screened, 1.75% hybridized with the C κ probe. Three clones were sequenced and found to be identical except for the length of the 5' untranslated region recovered (Fig. 2). This sequence began in the 5' untranslated region and included a normal-sized leader peptide and a V κ exon corresponding to the V κ IV subgroup gene (38) normally rearranged to the J κ 1 segment. The C κ region sequence was 100% identical to that of the normal Km(3) allele (39) (not shown). The rearranged V κ gene differed from the V κ IV subgroup germline gene by several single base substitutions, which resulted in nine amino acid changes: Tyr \rightarrow His (-2), Gln \rightarrow Leu (+27), Leu \rightarrow Phe (+27C), Tyr \rightarrow Phe (+27D), Ser \rightarrow Pro

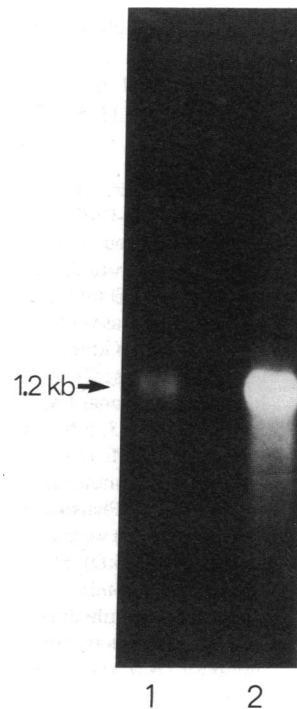


Figure 1. Northern blot analysis of RNA (5 μ g) hybridized with the C κ probe. (Lane 1) The control cell line JI producing normal sized κ mRNA. (Lane 2) Bone marrow cells from the LCDD patient.

Leader exon
-20
CAGGCAGGCAGGGGCGCAAG ATG GTG TTG CAG ACC CAG GTC TTC ATT TCT CTG
Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu

V_κ exon
-4 +1 +10
TTG CTC TGG ATC TCT GGT GCC CAC GGG GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT GTG TCT
Phe Leu Trp Ile Ser Gly Ala His Gly Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser
(Tyr)

+20 +27 A B C D E F +28 +30
CTG GGC GAG AGG GCC ACC ATC AAC TGC AAG TCC AGC CTG AGT GTT TTC TTC AGC CCC AAC AAT AAG AAC
Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Leu Ser Val Phe Phe Ser Pro Asn Asn Lys Asn
(Gln) (Leu Tyr) (Ser)

+40 +50
TAC TTA GCT TGG TAT CAG CAG AAA CCA GGA CAG CCT CCT AAG TTG CTC ATT TAC TGG GCA TCT ACC CGG
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg

+60 +70
GAA TCC GGG GTC CCT GAC CGA TTC AGT GGC AGC GGC TCT GGG ACA AAT TTC ACT CTC ACC ATC AGC CGC
Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asn Phe Thr Leu Thr Ile Ser Arg
(Asp) (Ser)

+80 +90 **J_{κ1} segment**
CTG CAG GCT GAG GAT GTG GCA GTT TAT TAC TGT CAG CAA TAT TAT ACT ACT CTT TCG TGG ACG TTC GGC
Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Leu Ser Trp Thr Phe Gly
(Ser) (Pro)

C_κ exon (Km3 allele)
CAA GGG ACC AAG GTG GAA ATC AAA CGA ACT GTG GCT GC...
Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala

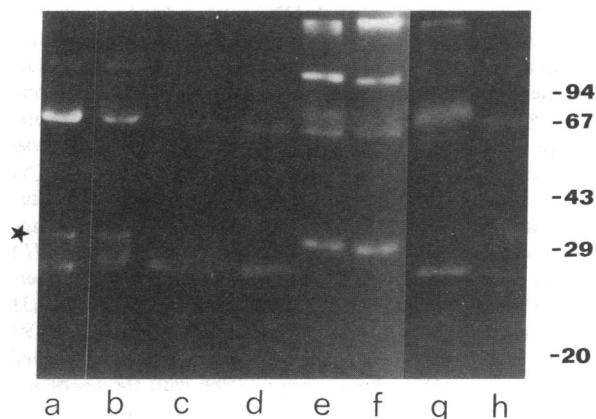
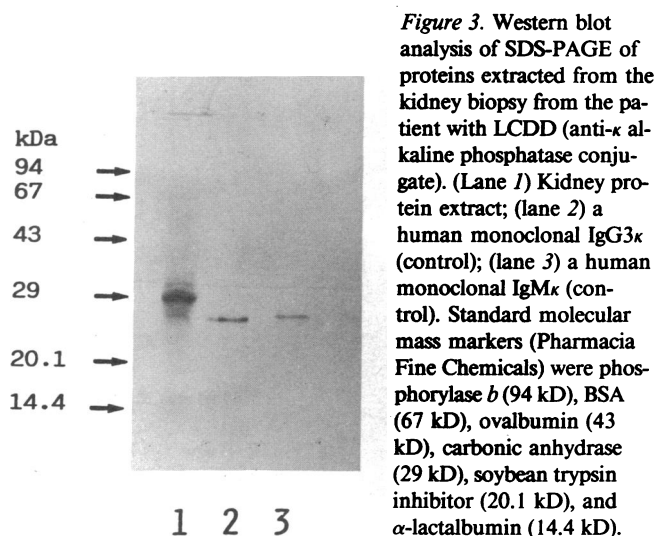
Figure 2. cDNA sequence of the variable region of the monoclonal κ chain produced by the LCDD patient's bone marrow plasma cells. Translation is indicated below the nucleotide sequence; differences in the germline V κ_{IV} gene-encoded sequence are indicated in brackets. The underlined aminoacid sequence (+1 to +13) is the sequence which was also found in the kidney κ chain. Numbering is according to Kabat et al. (35). These sequence data are available from EMBL/GenBank/DDBJ under accession number M38267.

(+27F), Asp \rightarrow Asn (+70), Ser \rightarrow Arg (+77), Ser \rightarrow Thr (+93), and Pro \rightarrow Leu (+95). The codon change at position +70 resulted in the creation of the sequence Asn-Phe-Thr which defines a potential *N*-glycosylation site. Computer simulation (kindly performed by Prof. J. D. Capra, Dallas, TX) did not suggest any major abnormality of the folding of the molecule.

Western blotting of proteins from the kidney biopsy specimen showed the presence of a single major κ band with an abnormally short migration (M_r 30,000) as compared with two different monoclonal κ chains (Fig. 3). Trace amounts of normal sized κ chains were also detected in the extract, as well as in the washing supernatants (not shown). Although the deposited κ chain represented apparently < 1% of the proteins fractionated by SDS-PAGE, the 13 NH₂-terminal aminoacids could be sequenced. This sequence was identical to residues determined by codons +1 to +13 of the cDNA (Fig. 2).

Study of biosynthetically labeled Ig produced by bone marrow cells is shown on Fig. 4. A band of the same M_r 30,000

as that of the kidney deposited κ chain was detectable in both cytoplasmic extracts and culture supernatants (Fig. 4, lanes *a* and *b*) of bone marrow cells labeled with ³H-leucine, together with a band of M_r 25,000 likely corresponding to κ chains from polyclonal B cells. The abnormally large κ chain was reduced to a normal size after treatment with *N*-glycosidase F (Fig. 4, lanes *c* and *d*). Only the enlarged κ chain was detectable among proteins labeled by incorporation of ¹⁴C-glucose (Fig. 4, lanes *e* and *f*). Molecules with M_r corresponding to γ , α , and μ heavy chains also incorporated ³H-leu and ¹⁴C-glucose and were coprecipitated by the anti- κ antibody, confirming the presence of a significant amount of polyclonal B cells. A control experi-



ment with the IgA1 κ producing cell line IARC317 showed that, under the same conditions, the α 1 heavy chain was also deglycosylated after *N*-glycosidase F treatment as expected, whereas the migration of the unglycosylated normal sized κ chains was unchanged (Fig. 4, lanes *g* and *h*). SDS-PAGE analysis of unreduced anti- κ precipitates showed that the patient's κ chain was mainly present in monomeric and dimeric forms in both cytoplasmic extracts and cell supernatants (not shown).

Discussion

The present patient presented with typical κ LCDD, and "non-secretory" myeloma. κ chains deposited in the studied kidney biopsy specimen were not significantly degraded, because virtually only a single band of the same abnormally high relative molecular mass as that of κ chains produced by bone marrow plasma cells was found on SDS-PAGE. Such an elevated apparent mass had been already demonstrated in other cases at the proliferative plasma cell level by biosynthetic labeling experiments (3, 4, 26–28). In addition to their similarly high relative molecular mass, identity between the deposited κ chain and that produced by the proliferating plasma cells was confirmed by the sequence of the myeloma cells κ mRNA and of the 13 NH₂-terminal amino acids of the kidney LC. The κ mRNA from the monoclonal bone marrow cells had a normal size by Northern blotting and sequencing and an overall normal structure. However, the V segment showed several peculiarities. It can be undoubtedly assigned to the V κ _{IV} subgroup. This subgroup is encoded by a single germline V gene (38) and accounts for ~8% of monoclonal κ chains (21). Several point mutations were found which could modify the conformation of the V domain. Four substitutions were observed in the complementarity determining region (CDR)1 at positions +27, +27C, +27D, and +27F. Two substitutions in the CDR3 included replacement of proline +95 by a leucine. Thus, both the CDR1 and CDR3 regions did not fit with any of the canonical structures defined for κ hypervariable regions (40). In particular, the presence of a proline residue at position +94 or +95 has been considered as being mainly responsible for the conformation of the third hypervariable region (41). Two substitutions were also identified in the framework (FR)3 region: Asp → Asn (+70) and Ser → Arg (+77), the former determining a potential *N*-glycosylation site at position +70. LC V regions of the V κ _{IV} subgroup have been entirely sequenced in five monoclonal Ig (42, 43); in none of these cases was a *N*-glycosylation site present. Evidence for glycosylation of the patient's κ chain was obtained from results of biosynthetic labeling with ¹⁴C-glucose. In addition, the abnormal size of the κ chains internally labeled with ³H-leucine shifted to normal after treatment with *N*-glycosidase F. This is in agreement with the strong PAS-positivity of the renal deposits. Glycosylated κ chains were found both in cytoplasmic extracts and cell supernatants, thus showing that *N*-glycosylation occurred in the bone marrow plasma cells, in agreement with our previous results in other patients. The single published LCDD κ chain of which the NH₂-terminal residues were sequenced belonged to the V κ _I subgroup (24), that is expressed in 56% of monoclonal κ chains (21). This κ chain, which was present in the deposits together with fragments, had *M_r* 28,000, which might relate to glycosylation also.

LCDD and AL amyloidosis are closely related diseases which may be associated in the same patient (4–6, 44–46). It may be of interest that glycosylated LC were found in amyloid

deposits (9, 11, 12). This is reminiscent of our previous finding in biosynthesis experiments of evidence suggestive of LC glycosylation, a relatively rare event in plasma cell proliferations (47), in every LCDD case with short or apparently enlarged LC (two-thirds of the studied cases, whereas in the other cases, monoclonal LC had a normal size without any data suggestive of glycosylation). We have also previously studied in more detail enlarged κ chains obtained from the serum from two patients with LCDD. These LC were found to contain 10.7 and 14.7% carbohydrates, respectively, with a composition compatible with *N*-glycosylation (4, 28). These LC were found to polymerize in vivo and in vitro by covalent and noncovalent bridges, possibly involving the presence of carbohydrates. Whether such a possible mechanism of LC polymerization might be involved in tissue deposition remains an open question. The present patient's κ chain was secreted mainly as monomer and dimer, and its sequence revealed no extra cysteine residue that could be used in disulfide bonds. Thus, glycosylation in itself might play a direct role in tissue precipitation because of binding to collagen and decreased catabolism of the light chains, as documented in a different situation, i.e., diabetes (48, 49). Such a hypothesis would explain the striking similarities between the glomerular lesions of LCDD and of diabetic nephropathy. As already mentioned, LC glycosylation is not a constant feature in LCDD. In the present patient, the unusual amino acid substitutions observed in the V κ region might modify its folding and conformation and favor tissue deposition. Computer simulation did not favor this hypothesis, but the limitations of this approach are well known. In any case and whatever the precise mechanism of deposition, the present observation documents a long-suggested but never-proven concept, i.e., that the basic pathogenetic abnormality in LCDD is at the LC level.

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