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Structural peculiarities of a truncated $V\kappa_{III}$ immunoglobulin light chain in myeloma with light chain deposition disease

C. DECOURT*, M. COGNÉ*† & A. ROCCA‡ **Laboratoire d'Immunologie et d'Immunogénétique, CNRS EP J0118, Faculté de Médecine and †Institut Universitaire de France, Limoges, and ‡Laboratoire d'Immunologie, CNRS URA 1172, IBMIG, Faculté des Sciences, Poitiers, France*

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SUMMARY

We report on the primary sequence of the monoclonal immunoglobulin light chain (LC) *REV* involved in myeloma-associated light chain deposition disease (LCDD). This sequence was deduced from that of the corresponding complementary (c)DNA in bone marrow plasma cells. Products of three independent amplifications by polymerase chain reaction (PCR) were sequenced and found to be identical. The κ mRNA encoding this N-glycosylated LC showed an overall normal structure consisting of a $V\kappa_{III}$ segment rearranged to $J\kappa_{II}$. Direct N-terminal amino acid sequencing of the circulating monoclonal IgA2,κ showed identity with the bone marrow-derived sequence. The κ-chain presented several unusual features affecting both the leader sequence and the variable (V) region. Four unique amino acid substitutions were found at positions -8, -3, -2 and -1 in the leader sequence and probably resulted in an unusual cleavage by signal peptidase, thus making the LC truncated by one residue and accounting for its unique hydrophobic N-terminus: Ile-Ile-Leu. Additional peculiarities were observed in the V region, including a Thr74 → Asn substitution creating a N-glycosylation site, and Thr53 → Ile, which was only reported once among human κIII chains, in another LCDD case, and may be of special significance at a position usually harbouring a polar amino acid.

Keywords light chain deposition disease $V\kappa_{III}$ subgroup κ chain

INTRODUCTION

Monoclonal immunoglobulin deposition diseases (MIDD) are severe complications of plasma cell dyscrasias, characterized by deposition in various organs of amorphous monoclonal immunoglobulin-related material, mostly light chain (LC), then termed LC deposition disease (LCDD). Although this condition is as yet poorly documented at the molecular level, various structural features have been suggested to correlate with the tendency of a given LC to aggregate and precipitate [1–11]: N-glycosylation [6]; exchange of polar residues by hydrophobic ones [5,9]; replacement of highly conserved residues supposedly involved in the maintenance of the LC structure [6,8]. Only four complete primary sequences of LC involved in LCDD have been published to date: two of the subgroup $V\kappa_{IV}$ [6,8], one of the subgroup $V\kappa_{I}$ [9], and one (*SCI*) of the subgroup $V\kappa_{III}$ [5]. A $V\kappa_{IV}$ LC (*BLU*) was N-glycosylated as a result of an Asp → Asn substitution at position +70 [6]. The others (*SCI*, *FRA*, *ISE*) did not have an N-glycosylation site [5,8,9].

We report on another patient with myeloma-associated LCDD (*REV*), for whom biochemical study and direct N-terminal amino acid sequence determination of the circulating monoclonal IgA2,κ had previously shown that the monoclonal κ-chain was N-glycosylated and began with a very unusual hydrophobic N-terminus (Ile-Ile-Leu) [10]. In the present study, we determined from bone marrow mRNA the complete sequence of the cDNA corresponding to this LC and its leader peptide in order to investigate the origin of its unusual N-terminus and N-glycosylation site and to look for other potential primary sequence peculiarities.

Correspondence: Dr Michel Cogné, Immunologie et Immunogénétique, CNRS EP J0118, Faculté de Médecine, F-87025 Limoges, France.

PATIENT AND METHODS

Patient (*REV*) was referred for myeloma. A monoclonal IgAκ was identified in the serum while the bone marrow aspiration showed the presence of 50% plasma cells. The patient then received 24 courses of chemotherapy with drugs including vincristin, adriamycin, melphalan, cyclophosphamide and prednisone, followed with treatment by interferon-alpha (IFN-α). Three years later, this patient was referred for renal failure. No κ type Bence Jones protein was detectable in the urine. Study of a kidney biopsy by light microscopy and immunofluorescence showed nodular glomerulosclerosis and monotypic κ-chain deposits along tubular and glomerular basement membranes and in arterial walls. The

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deposits were periodic-acid-Schiff-positive and Congo red-negative. By immunofluorescence, the deposited material stained with anti-κ but not with anti-α, μ, γ or λ conjugates. Electron microscopy confirmed the presence of amorphous granular deposits along the outer part of the tubular basement membranes, and pathological examination was thus typical of myeloma-associated LCDD.

Total RNA was prepared by lysis of bone marrow cells in 4 M guanidine isothiocyanate followed by centrifugation at 170 000 g for 18 h on a 5·7 M caesium chloride pad. Total RNA was analysed on a 1% agarose, 0·7 M formaldehyde gel in comparison with RNA from the human lymphoma cell line *IARC*518 producing normal-sized κ mRNA and from the plasmacytoma cell line *RPMI*8226 producing a normal-sized λ mRNA [12], transferred to nylon sheets and hybridized with either a Cκ probe, a 2·5-kb EcoRI genomic fragment containing the human Cκ exon, or a Cλ probe, a 3·5-kb EcoRI–Hind3 fragment containing the human Cλ2 exon. Poly(A) mRNA was prepared by affinity chromatography on oligo(dT)-cellulose (Pharmacia, Uppsala, Sweden) and was used as template for synthesizing single-stranded cDNA using reverse transcriptase (RT) and an oligo(dT) primer (Boerhinger, Mannheim, Germany). Polymerase chain reaction (PCR) primers were: a $5'$ primer corresponding to a Vκ_{III} leader region (ATGGAAACCCCAGCGCAGCTT) and $3'$ primers complementary either to the upstream (GCGGGAAGATGAAGACAGATGGTGCAG) or to the downstream part of the Cκ exon (CTGGAACTGAGGAGCAGGT). Amplification of the cDNA by PCR was performed with Taq polymerase (Cetus, Emeryville, CA) through 35 cycles consisting of: denaturation at 94°C for 30 s, annealing at 63°C for 30 s and elongation at 72°C for 30 s [13]. After amplification, PCR products were fractionated on 1·0% agarose gels and cloned in SmaI cut M13 mp19 vector or in SmaI cut pBluescript vector. Sequencing was performed by the dideoxy termination method [14] using T7 polymerase and an automated laser fluorescent DNA sequencer (Pharmacia).

RESULTS

Using bone marrow cell RNA in a case of myeloma-associated LCDD, we studied mRNAs by Northern blotting and isolated cDNA clones through RT and PCR experiments. Northern blots hybridized with a Cκ probe yielded a very strong signal of a normal size (Fig. 1), indicating that the bone marrow was infiltrated by a high proportion of homogeneous κ producing cells. When the amount of non-malignant polyclonal B cells was estimated through hybridization of the same Northern blot with a Cλ probe, λ-producing polyclonal B cells did not show up and there were no detectable λ transcripts in the bone marrow.

Products of five independant RT-PCR amplifications of either the Vκ region or of the complete κ cDNA were cloned and sequenced. Nucleotide sequences obtained from three RT-PCR experiments were identical and the deduced amino acid 2–19 sequence fell in complete agreement with the 18 N-terminal amino acid sequence of the serum monoclonal IgA2-bound κ-chain (Fig. 2): it was thus possible to assign the deduced amino acid sequence to the monoclonal κ-chain *REV* produced by the malignant bone marrow plasma cells. In two other RT-PCR experiments, variant sequences were found which differed from that of the monoclonal serum protein and which, given the high amount of malignant plasma cells in the bone marrow, corresponded to minor subclones of the malignant cells rather than to mRNA from polyclonal B cells; these variant mRNA also belonged to the Vκ_{III} subgroup, one of them (*REV25–3*) was rearranged to the same Jκ2 fragment as *REV25–2*, whereas the other PCR fragment (*REV33–9*) was truncated and its J region not sequenced (Fig. 3).

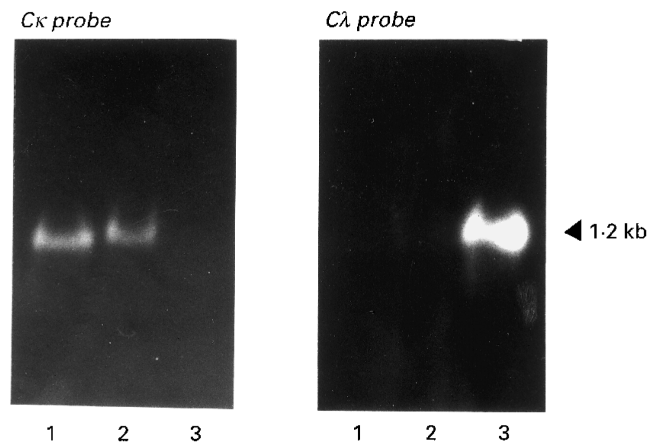

Fig. 1. Northern blot analysis of bone marrow total RNA. RNA from patient *REV* bone marrow cells (lane 1) was hybridized with either a human Cκ probe and compared with the normal κ mRNA from the *IARC*518 cell line (lane 2) or a human Cλ probe and compared with the normal λ mRNA from the *RPMI*8226 cell line (lane 3). Sizes are indicated in kilobases.

DISCUSSION

The *REV25–2* sequence corresponding to the circulating monoclonal protein was made up of a Vκ_{III} subgroup V exon [15] normally rearranged to the Jκ2 segment [16]. In another LCDD case, direct amino acid sequencing of the Bence-Jones protein also identified a Vκ_{III} subgroup LC (protein *SCI*) [5]. When the *REV* sequence was compared with known Vκ_{III} germ-line sequences [17], the best homology (94·4%) was found with the germ-line gene L2. It is worth noting that *SCI* LC was also related to the same family of germ-line genes, family κIIIa, which is often expressed in autoantibodies with rheumatoid factor activity [18,19]. Alignment of the N-terminal amino acid sequence of protein *REV* with known protein sequences showed that it was shorter by one residue than all previously reported κ-chains and begun with the normal Ile2 residue of the Vκ_{III} subgroup (according to Kabat's numbering [15]). Absence of Glu1 makes the chain begin with a stretch of three hydrophobic residues (Ile-Ile-Leu). However, the codon for Glu1 was not deleted in the cDNA sequence; rather, amino acid substitutions occurred in the leader region, which differed from the germ-line L2 sequence at four positions: Leu(-8) → Val; Thr(-3) → Met, Thr → (-2)Ser and Gly(-1) → Glu. It is thus tempting to hypothesize that the amino terminal deletion resulted from a variation in the cleavage site chosen by signal peptidase, whose specificity relies on the sequence of residues -5 to -1 [20,21]. Since positions -3 and -1 are known to play a crucial role in the processing by signal peptidase, substitutions in *REV* at those positions could lead to a + 1 shift in the recognition and cleavage site.

In addition to its abnormal N-terminus, the *REV* LC presented 10 unusual residues in the V region, most of them located in the FR regions. Among the two unusual amino acids located in the CDRs, Ile53 corresponded to a substitution previously unique to the Vκ_{III}

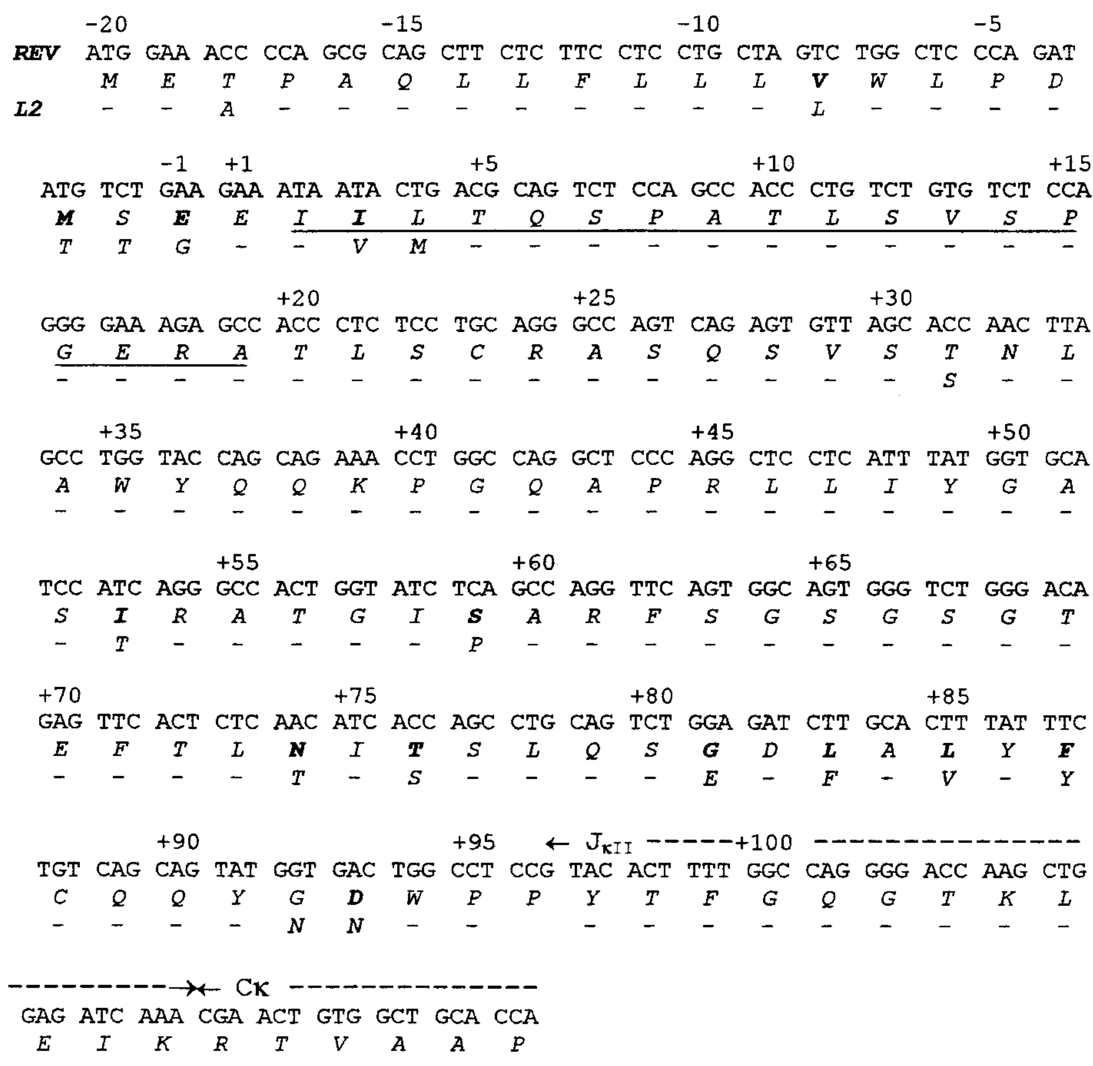

Fig. 2. Sequence of the *REV25−2* mRNA corresponding to the monoclonal serum κ-chain. The nucleotide sequence is indicated together with the deduced amino acid sequence. N-terminal amino acids determined from the serum monoclonal immunoglobulin κ-chain are underlined. Lower line indicates the sequence encoded by the germ-line L2 and Jκ2 genes, dashes indicate identities, unusual residues are in bold.

LCDD protein *SCI* [5]. This residue is located in a region always harbouring polar amino acids, most often Ser, in κ LC. Furthermore, residue 53 has been proposed to play a key role in the conformation of the antigen binding loop L2 [22]. The Ser → Ile substitution may thus impose a local structural alteration of the CDR2, which may relate to the tendency for LC deposits.

Interestingly, another unusual substitution, Asp93, located in the CDR3, is also shared by *REV* and *SCI* LCs and has only been previously described for three LC of the $V\kappa_{III}$ subgroup [15]. All other eight amino acid differences were in FR regions and especially in the FR3, which harboured seven out of the 10 unusual residues in LC *REV*, including Thr74 → Asn that created the potential N-glycosylation site: Asn-Ile-Thr. That position 74, the only potential site in the protein, was indeed N-glycosylated, was known from SDS−PAGE analysis with or without N-glycosidase F treatment [10]. Another LCDD LC (*BLU*), belonging to the $V\kappa_{IV}$ subgroup [6], was also featured by a N-glycosylation site in the FR3 region. In fact, N-glycosylation is common in LCDD cases and has been proposed to play a role in LC deposition [6,7] as well as in the related deposition disease, AL-amyloidosis [23−25]: glycosylation by itself might play a direct role in tissue precipitation through interactions of carbohydrates with components of the extracellular matrix [26,27].

Among the other substitutions in *REV*, Ile3 and Gly81, located in the FR1 and FR3 regions, had never been reported at these positions in the $V\kappa_{III}$ subgroup. Ser59, Leu83, Leu85 and Phe87, all located in the FR3 region, have been rarely reported in human κ-chain sequences. Some of these unusual substitutions (Ile3, Thr31, Ser59, Leu83, Leu85) alter highly conserved residues presumed to be important for LC conformation or monomers interaction [28].

Another issue of the present case is the putative relationship between the occurrence of the deposition disease and the pursued chemotherapy. The patient was first referred for myeloma without renal involvement but developed renal failure with LCDD 3 years later. During those 3 years, the patient received several chemotherapy cycles with drugs such as melphalan, potentially able to induce mutations in immunoglobulin genes [29]. It might thus be possible

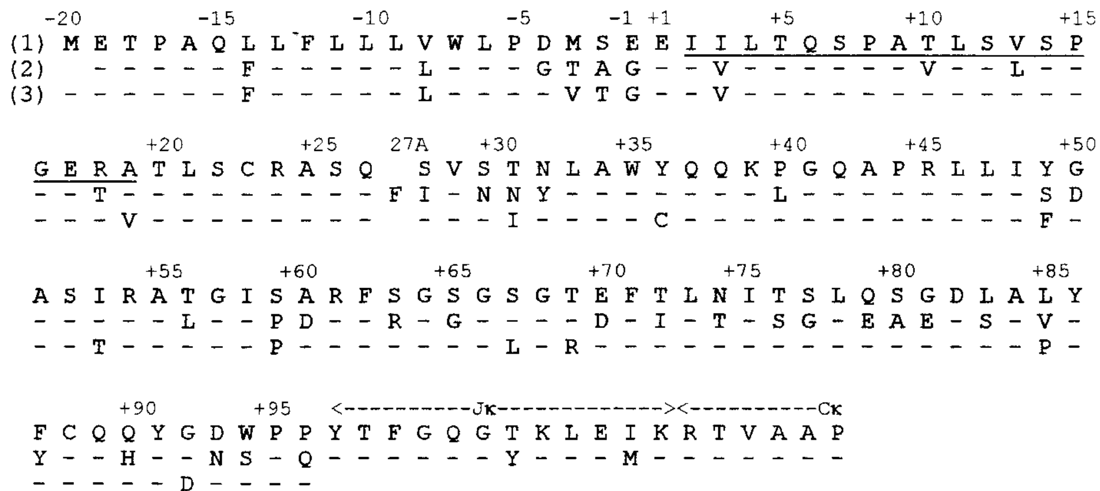

Fig. 3. Comparison of protein sequences encoded by the predominant mRNA *REV25−2* (1) in patient *REV* bone marrow and by the two variant mRNA *REV25−3* (2) and *REV33−9* (3). N-terminal amino acids determined from the serum monoclonal immunoglobulin κ-chain are underlined. Dashes indicate identities.

that some unusual amino acids of LC *REV* resulted from chemotherapy-induced gene mutations. Such a natural history of the disease would be reminiscent of other previously reported LCDD cases with onset after the initiation of chemotherapy by alkylating agents [2,3]. It would also fit with the evidence of variant κ mRNA sequences produced by the *REV* malignant plasma cells (Fig. 3), which is a likely indication that several variant subclones synthesizing immunoglobulin mRNA with divergent sequences have evolved from the initial malignant cells. Finally, for the predominent *REV25−2* mRNA sequence corresponding to the serum monoclonal immunoglobulin, it is not currently possible to ascertain whether some peculiar substitutions result from genetic polymorphism, antigen-driven somatic mutations, or chemotherapy induced mutations. Whatever the origin of substitutions, a clear comprehension of the disease will probably await tri-dimensional structure analysis of pathogenic proteins, site-directed mutagenesis of nephrotoxic sequences and accumulation of new primary sequence data about non-nephrotoxic LCs *versus* nephrotoxic LCs involved in LCDDs.

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