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Overrepresentation of the $V\kappa_{IV}$ subgroup in light chain deposition disease

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1. Summary

The variability subgroup of human monoclonal κ chains purified from urine in 3 consecutive patients with myeloma associated light chain deposition disease was determined from amino acid sequences of their first framework regions (FR1). N-glycosylation was searched for by N-glycosidase F treatment. These data together with our previously published results, indicate the pathogenic potential of the rare $V\kappa_{IV}$ subgroup and confirm the absence of detectable serum and urine free monoclonal light chains when they are N-glycosylated.

2. Introduction

Non-amyloid monoclonal immunoglobulin (Ig) light chain (LC) deposition disease (LCDD) is a severe complication of plasma cell dyscrasias, either apparently benign or malignant. It occurs in about 5% of myeloma cases. LCDD affects constantly the kidney and frequently involves other vital organs such as heart and liver as well. Visceral deposition of LC (mostly of the κ type) derived material occurs in certain extracellular areas, particularly along basement membranes [1]. This material differs from amyloid deposits by its granular, non-organized ultrastructure, absence of birefrigence under polarized light after staining with reagents such as Congo red or thioflavine T and of detectable amyloid P-component. Unusual size, polymerization, and/or glycosylation have been correlated with the propensity of monoclonal Ig LC to cause LCDD [reviewed in 2]. The pathogenic role of structural alterations intrinsic to monoclonal LC in deposition diseases has been confirmed in experimental animals [3]. However, primary structure data did not allow demonstration of well-defined common features. Isotype restriction is clear in LCDD, with κ chains occurring in approximately 80% of cases [1], as well as in AL-amyloidosis where the λ isotype is largely predominant [4]. In the latter condition, a rare V region variability subgroup, $V\lambda_{VI}$, displays striking amyloidogenic properties [5]. This led us to further study $V\kappa$ subgroups in LCDD. We previously established the primary structure of monoclonal κ chains in 3 consecutive LCDD cases [6–8]. Strikingly, two of them belonged to the $V\kappa_{IV}$ subgroup, which is rarely expressed in myeloma.

In the present study, we collected kappa LC in 3 further cases of LCDD and determined their N-terminal sequence, to confirm the prevalence of the $V\kappa_{IV}$ subgroup. N-glycosylation was also searched for and found only in patients without detectable circulating free monoclonal LC.

3. Material and Methods

The 3 patients presented with proteinuria and renal failure. Study of kidney biopsies by light microscopy and immunofluorescence showed nodular glomerulosclerosis and monotypic κ chain deposits along tubular and glomerular basement membranes and in arterial walls. In patient MUL, a faint staining for α chain was also observed in the deposits. In one case (REV) the diagnosis was confirmed by electron microscopy. Monoclonal Ig were searched for in the patients' serum and concentrated urine by immunoelectrophoresis and immunoblotting after thin layer agarose electrophoresis.

A monoclonal IgA κ and free κ chains were partially purified from concentrated urines of patient MUL by Sephadex G100 (Pharmacia, Uppsala, Sweden) chromatography followed by preparative agarose (low electroendo-osmosis, Sigma, Saint-Louis, MO) elec-

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trophoresis in 50 mM barbital buffer pH 8.6. Urinary free κ chain from patient DOS was isolated by gel filtration on Ultrogel AcA34 (Sepracor, Villeneuve-la-Garenne, France) followed by DEAE-chromatography using the memSep System (Millipore, Bedford, MA) in 20 mM TRIS buffer pH 8.2 with a 0 to 0.3 M NaCl linear gradient. A monoclonal IgA2 κ was partially purified from patient REV's serum by DEAE-Trisacryl (Sepracor) chromatography in 10 mM TRIS pH 7.6 with a 0 to 0.3 M NaCl linear gradient; residual polyclonal IgA1 were removed by adsorption on jacalin-Sepharose 4B.

N-glycosylation was searched for, after denaturation of the protein samples in 0.5% sodium dodecyl sulfate at 100°C during 2 mn, by treatment with N-glycosidase F (Boehringer, Mannheim, Germany, 1 unit for 0.5 mg proteins) in 20 mM sodium EDTA 2 mM phenyl-methyl-sulfonyl fluoride 0.1 M TRIS pH 8.6 at 37°C during 2 hours. Samples were analysed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) in comparison with the native proteins.

For N-terminal sequencing, reduced samples were fractionated by 12% SDS–PAGE in 10 mM reduced glutathione (Sigma) and transferred onto Immobilon P membranes (Millipore) according to Matsudaira [9]. Sequence determinations were performed by Edman degradation using a 470A Applied Biosystems (Foster City, CA) gas-phase sequencer [10]. Phenylthiohydantoin (PTH) amino acids were identified on line with a 120A Applied Biosystems PTH-Analyzer by reversed-phase HPLC. Sequencing was performed using the 03RPTH program with slight modifications. Chemical delivery rates were carefully monitored at the beginning of each sequence determination.

4. Results

N-terminal sequences of the patients' LC are presented in Table 1, in comparison with consensus structures deduced from the Kabat database [11]. The κ chain MUL sequence was identical in the monoclonal IgA and Bence-Jones protein and belonged to the $V\kappa_{III}$ subgroup; residue Thr at position 2, which differs from the consensus sequence, has been found in two proteins (VER and K-EV15) of this subgroup [11]. The κ chain REV also belonged to the $V\kappa_{III}$ subgroup and it was featured by an unusual hydrophobic N terminus lacking the first amino acid. Sequence homology allowed to assign protein DOS to the $V\kappa_{IV}$ subgroup. Comparisons with EMBL data bank sequences using program FASTA confirmed that the most homologous proteins belonged to the $V\kappa_{III}$ subgroup for MUL and REV and to the $V\kappa_{IV}$ subgroup for DOS.

TABLE I

N-TERMINAL AMINO ACID SEQUENCES OF κ CHANS FROM 3 LCDD PATIENTS

Consensus sequence corresponding to variability subgroups ($V\kappa_I$ to $V\kappa_{IV}$) are indicated in the lower part of the table.

Residue N°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
protein origin																				
MUL IgA κ	E	T	V	M	T	Q	S	P	A	T	L	S	V	S	P	G	E	R	A	T
MUL BJ κ	E	T	V	M	T	Q	S	P	A	T	L	S	V	S	P	G	E			
REV IgA κ	–	I	I	L	T	Q	S	P	A	T	L	S	V	S	P	G	E		A	
DOS BJ κ	D	I	V	M	T	Q	S	P	E	S	L	A	V	S	L	G	E	R		
$V\kappa_I$	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T
$V\kappa_{II}$	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A	S
$V\kappa_{III}$	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	P	G	E	R	A	T
$V\kappa_{IV}$	D	I	V	M	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T

N-glycosylation was revealed by a shift in the migration on SDS–PAGE of the κ chain REV after N-glycosidase treatment; by contrast, electrophoretic mobilities were not affected by similar treatment of κ chains MUL and DOS (not shown). Data on circulating monoclonal Ig, N-glycosylation and V region subgroups in the 6 consecutive patients studied in our laboratory, including published cases [6–8] are shown in Table 2. The incidence of Vκ subgroups was compared with that reported in 157 myeloma cases [12], using the χ^2 test with the Yates' correction: in spite of the small number of cases, a significant ($P < 0.003$) overexpression of the $V\kappa_{IV}$ subgroup features LCDD.

Both N-glycosylated κ chains (REV and BLU) were undetectable in the patients' serum and urine in contrast to the patients with no evidence of LC glycosylation.

5. Discussion

The present study suggests that monoclonal κ chains of the $V\kappa_{IV}$ subgroup are more prone to cause LCDD than others. Although significantly higher than in myeloma patients without known deposition disease, the precise incidence of this subgroup should be determined in a larger series of LCDD patients. We did not include in this study the two other cases published so far (McM [13] and kSci [14], which belonged to the $V\kappa_I$ and $V\kappa_{III}$ subgroups, respectively) because we believe that a homogeneous recruitment is prerequisite when looking at the incidence of LC structural peculiarities in this condition [2]: proteins McM and kSci were analysed at the molecular level because large amounts could be purified, which is not the case in all LCDD patients [2,6]. However, even when they are taken into account, $V\kappa_{IV}$ subgroup overexpression remains significant ($P < 0.02$).

The $V\kappa_{IV}$ variability subgroup is encoded by a single germline V segment proximal to the Jk cluster. Its overall incidence among myeloma Bence-Jones proteins is lower (7% of cases) [12] than in adult normal peripheral B lymphocytes [15]. The present data point for an unusual Vκ repertoire in LCDD. This is in agreement with the hypothesis of a linkage between a yet unidentified antibody-like reactivity of LC and their propensity to deposit close to certain extracellular matrix (EM). Studies are presently under progress in our laboratory to look for reactivities of these LC with EM components.

The role of N-glycosylation in LC deposition may be important in view of its increased frequency in LCDD [2]. We previously sequenced a N-glycosylated kappa chain in kidney deposits and plasma cells from a myeloma-associated LCDD patient (BLU) without detectable circulating LC, although myeloma plasma cells were shown to synthesize free LC in vitro [6]. Among the 3 other cases of the present study, a N-glycosylated monoclonal LC was found only in patient REV who had no detectable free LC in the serum and urine, confirming previous data from our group [reviewed in ref. 2]. As previously suggested [2], LC glycosylation might be a factor enhancing tissue deposition, which could explain the lack of detection of the free circulating form.

In conclusion, the present study suggests that, beside molecular biology analyses, features such as N-glycosylation, V region repertoire and binding properties may help predicting and understanding of LC pathogeneticity.

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TABLE II

MAIN FEATURES OF CIRCULATING AND DEPOSITED MONOCLONAL Ig IN 6 PATIENTS WITH MYELOMA ASSOCIATED LCDD.

Patient	Tissue deposited Ig	Monoclonal Ig		Monoclonal LC N-glycosylation	Vκ Subgroup
		Serum	Urine		
ISE [8]	κ	κBJ *	κBJ	–	I
MUL	α, κ	IgA κ	κBJ, IgA κ	–	III
REV	κ	IgA κ	–	+	III
DOS	κ	IgG κ	κBJ, IgG κ	–	IV
BLU [6]	κ	–	–	+	IV
FRA [7]	κ	IgG κ	κ BJ, IgG κ	–	IV

* BJ: Bence-Jones protein.

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