RAPID COMMUNICATION

Role of Light Chain Variable Region in Myeloma With Light Chain Deposition Disease: Evidence From an Experimental Model

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Light chain deposition disease (LCDD) results from a propensity of some human monoclonal L chains to form tissue deposits. We designed an experimental model for in vivo expression of human κ L chain sequences in mice and compared a somatically mutated LCDD chain with a closely related control κ chain, both encoded by the unique VκIV gene. Mice secreting the LCDD chain but not those producing the control chain showed deposits with a distribution similar to that observed in patients. These data show that discrete changes in V region sequences can play a major role in tissue deposition of human L chains.

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NONAMYLOID monoclonal Ig deposition disease (MIDD) is a severe complication of human immunoproliferative disorders, characterized by tissue deposition of monoclonal Ig chains, with a prominent kidney impairment.1,2 The occurrence of MIDD in myeloma is close to 5% of cases. MIDD deposits lack the staining properties and fibrillar organization of AL-amyloidosis, but exhibit granular material by electronmicroscopy. By immunofluorescence, an homogeneous staining is observed along basement membranes and in the glomerular mesangium. Deposits may contain only light (L) chain determinants in L chain deposition disease (LCDD), both L and heavy (H) chain determinants (LHCDD), or H chain determinants alone (HCDD).1,4

Since the first description of LCDD,5 convergent data suggested the role of structural abnormalities of Ig chains in tissue deposition, including abnormalities in size, glycosylation, and/or variable (V) region sequence peculiarities.1,2,6 Moreover, the injection of purified human Bence Jones (BJ) proteins into mice yielded kidney deposits, showing the nephrotoxic potential of some BJ proteins.7,8 Recently, the elucidation of Ig sequences in several MIDD patients showed V region peculiarities.9,10,12 In the aim of precisely defining the role of V domain sequences in L chain nephrotoxicity, we set up an experimental model in which human L chains with particular V regions could be secreted in vivo in mice. The κ chain FRA was originally identified in a patient with myeloma and LCDD10 and belonged to the VκIV subgroup, which is encoded by a single germline gene11 apparently overrepresented in LCDD.14 Our model allowed comparison between that pathogenic chain and a related chain of the same VκIV subgroup, showing that some substitutions in the L chain V region are sufficient to promote tissue deposition.

MATERIALS AND METHODS

Production of human L chains in mice in vivo. The sequence of the LCDD chain FRA, encoded by a rearranged VκIV-Jκ1 gene, was previously cloned as a cDNA.10 Sp2/0, an Ig nonproducing B-cell hybridoma, was transfected with plasmid pAKMC.15 carrying the sequence of the cDNA FRA and driven by a VH promoter and a combination of the human and murine intronic Ig HC enhancers. Alternatively, for the expression of the control chain, Sp2/0 cells were transfected with pAKKeI,15 carrying rearranged genomic VκIV and Jκ4 segments (kind gift of Dr H.G. Klokeb, Institut für Physiologische Chemie, Munich, Germany). Transfection of Sp2/0 and analysis of clones with the highest expression were as described.15 Untransfected Sp2/0 cells or selected transfecoma clones with similar in vitro secretion (1.3 μg/mL/24 h/10⁶ cells) of either κ chain were injected into 8- to 10-week-old BALB/c mice (5 × 10⁵ cells in 0.5 mL of phosphate-buffered saline). Mice injected with untransfected Sp2/0 cells are thereafter called UT mice. Those injected with transfecoma clones producing either the LCDD FRA chain or the control κ chain are called F and C mice, respectively. Mice were injected intravenously (into a tail vein) rather than intraperitoneally because the former procedure yielded retroperitoneal tumors and a longer survival than intraperitoneal tumors (mean, 10 ± 6 weeks).

Immunohistochemical studies. For Western blot, urine samples were collected just before the killing of the mice, analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (a SDS-PAGE), transferred to nylon sheets, and shown with a biotinylated monoclonal antihuman κ and streptavidin alkaline phosphatase conjugate (Amersham, Buckinghamshire, UK). L chain secretion levels were measured in the serum by enzyme-linked immunosorbent assay (a ELISA), as described.15 Human κ chains were undetectable in UT mice but consistently detected in F and C mice.

Immunohistochemical studies. Light microscopic examination was performed after staining with hematoxylin and eosin, periodic acid Schiff, silver methenamine, or toluidine blue. Frozen blocks of all major organs obtained at killing of mice were cut in 4-μm-thick slices. Organs were studied for the deposition of human and mouse Ig by immunofluorescence with fluorescein-conjugated polyclonal rabbit antihuman κ or λ chain (Dako, Glostrup, Denmark) or with rabbit antigoat Ig-rhodamine conjugate (Jackson Immunoresearch, West Grove, PA).

Parts of the kidneys were stained with osmium for ultrastructural examination with a JEOL 100CX electron microscope (Japan Electron Optical Laboratory, Tokyo, Japan).

Northern blot analysis. Total RNAs (10 μg) from untransfected Sp2/0 cells, transfecoma, mice tumors, and the patient FRA’s bone marrow were analyzed on 7% formaldehyde–1.2% agarose gels, stained with ethidium bromide, and examined by UV transillumination. Transfection of Sp2/0 cells and analysis of clones with the highest expression were as described.15

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transferred to nylon sheets, and hybridized with a probe corresponding to the FRA cDNA.\textsuperscript{10}

\textit{Polymerase chain reaction (PCR) and sequence analysis.} Total RNAs from F3, F7, and C9 mice tumors were studied by reverse transcription and PCR with a 5' primer corresponding to V\(\kappa\)IV leader region (ATGGTGTTGCAGACCCAG) and a 3' primer complementary to the 3' untranslated region of \(\kappa\) (CTGGAACTGAGGAGC-AGGT).\textsuperscript{16} PCR products were cloned and sequenced by the dideoxynucleotide method.\textsuperscript{17}

\section*{RESULTS}

A high secretion rate of human \(\kappa\) chains was obtained in vitro through transfection of Sp2/0 hybridoma cells with expression vectors carrying the sequences of either the LCDD \(\kappa\) chain (FRA) or the control \(\kappa\) chain. The LCDD \(\kappa\) chain FRA was not glycosylated and its V region (residues 1 to 95) bore 14 aminoacid substitutions if compared with the single V\(\kappa\)IV subgroup germline gene\textsuperscript{10,13,18} (Fig 1). The V\(\kappa\)IV segment encoding the control chain differed from the germline by 3 substitutions\textsuperscript{13} (Fig 1). In vivo production was obtained through injection of mice with either \(\kappa\) chain FRA or control L chain transfectedomas (F and C mice, respectively), but not with untransfected Sp2/0 cells (UT mice). Two groups of 12 F mice or 8 C mice were compared for secretion of \(\kappa\) chains in the serum and formation of L chain deposits in the kidneys at death, 8 to 12 weeks after injection (Table 1).

By immunofluorescence, human \(\kappa\) chain determinants were readily detected along basement membranes in tumors and in every organ studied (kidney, liver, spleen, ovary, and heart) in 11 of 12 F mice (Fig 2B [kidney] and Fig 2C [liver]), with a staining pattern very similar to that obtained in patient FRA (Fig 2A). By contrast, no deposit was detected in any organ of the 8 C mice (Fig 2D) and 4 UT mice (data not shown). Lack of deposits in one F mouse (F3) likely related to its short survival after injection (5 weeks),

\begin{table}[h]
\small
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\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Mice} & \textbf{Serum \(\kappa\) Chain (\(\mu\)g/mL)} & \textbf{Survival (wk)} & \textbf{Kidney Deposits (immunofluorescence)} \\
\hline
F3 & 70 & - & - \\
F5 & 64 & 11 & + \\
F7 & 32 & 10 & + \\
F8 & 22 & 10 & + \\
F9 & 11 & 11 & + \\
F10 & 66 & 10 & + \\
F11 & 28 & 12 & + \\
F12 & 9 & 10 & + \\
F13 & 66 & 12 & + \\
F14 & 30 & 10 & + \\
F30 & 32 & 11 & + \\
F31 & 128 & 10 & + \\
C2 & 21 & 10 & - \\
C3 & 29 & 10 & - \\
C4 & 74 & 11 & - \\
C5 & 2.8 & 10 & - \\
C6 & 2 & 10 & - \\
C7 & 4.7 & 12 & - \\
C8 & 20 & 10 & - \\
C9 & 80 & 11 & - \\
\hline
\end{tabular}
\caption{Human \(\kappa\) Chain Secretion in Serum, Survival Duration, and Presence or Absence of \(\kappa\) Chain Deposits by Immunofluorescence In Kidneys From F and C Mice}
\end{table}
IMMUNOFUORESCENCE STUDY OF KIDNEY SAMPLES USING FITC-LABELED POLYCLONAL ANTI-HUMAN κ CHAIN ANTIBODIES. (A) LCCD patient FRA. (B) A representative F mouse (F7) kidney with an homogeneous staining along the capsular basement membrane, glomerular capillaries, and arteriole. (C) A representative F mouse (F7) liver with staining in the space of Disse and centrilobular vein. (D) A representative C mouse kidney (C9) with human κ chains being internalized by the proximal tubular cells. No staining along any basement membrane could be detected.

whereas all other mice survived longer than 10 weeks. In C mice, small spots in kidney proximal cells indicated an internalization of the control chain (Fig 2D), suggesting a normal reabsorption. Staining with the anti-λ conjugate was negative in all mice (data not shown). By contrast to immunofluorescence, we were unable to detect any lesion or deposit in the kidneys from F, C, or UT mice by light and electron microscopy.

Identity of L chains produced in mice with the expected translation products of the expression vectors was checked at the mRNA and protein levels. Northern blotting of total RNAs from transfectomas grown in vitro or from tumors showed κ mRNAs of the expected size in view of the splice sites used in the vectors (Fig 3). No human κ mRNA was detected in UT mice tumors. Western blot of urines of F and C mice showed normal-sized chains (data not shown). The absence of any mutation in transfected genes during tumoral growth in mice was checked by sequencing κ cDNAs from representative F (F3 and F7) and C (C9) mice (data not shown).

DISCUSSION

We have established a partial murine model of human LCDD in which only those mice expressing a nephrotoxic
κ chain from a patient with myeloma and LCDD had detectable deposits by immunofluorescence. Contrasting with immunofluorescence data, nodular glomerulosclerosis and lesions typical of MIDD were undetectable by light or electron microscopy, which might also relate to the short survival of injected mice (10 to 12 weeks), and/or to insufficient secretion rates. Indeed, in contrast to L-chain-related primary amyloidosis, a significant plasma cell mass seems to be required for clinical expression of MIDD, and in two patients in whom LCDD likely resulted from mutations induced by alkylating agents, the first symptoms of the disease occurred 8 and 12 months after chemotherapy, respectively. The herein reported murine LCDD model thus likely mimics findings in certain human LCDD patients in whom the diagnosis of LCDD was based on functional alterations of the kidneys and typical LCDD staining by immunofluorescence, in the absence of detectable morphologic lesions. A likely hypothesis is that glomerulosclerosis occurs later in the time course of the disease. F mice might then be of interest to study an early stage of LCDD of which they are representative.

L chains are of the κ type in 80% of LCDD patients, with a probable overrepresentation of the VκIV subgroup. Our data confirm that not all VκIV L chains are similarly prone to form deposits when present at similar concentrations; similarly to our control κ chain, the κIV protein LEN in the kidney and plasma cells in light chain deposition disease. J Biol Chem 266:293, 1991

References

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