Bence Jones Proteins and Light Chains of Immunoglobulins

PREFERENTIAL ASSOCIATION OF THE $V_{\lambda VI}$ SUBGROUP OF HUMAN LIGHT CHAINS WITH AMYLOIDOSIS AL(λ)

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ABSTRACT An antiserum prepared against a λ-Bence Jones protein from a patient (SUT) who had multiple myeloma and amyloidosis had specificity for λ-light chains of the chemically defined variable (V) region λ-chain subgroup λVI. Sequence analyses of protein SUT and of five other λ-light chains recognized immunologically as of the $V_{\lambda VI}$ subgroup revealed that all six proteins had the N-terminal sequence characteristic for prototype λVI proteins. The isotypic nature of the $V_{\lambda VI}$ subgroup was demonstrated immunochemically: λVI molecules were detected among light chains isolated from the IgG proteins of each of 12 normal individuals and λVI antigenic determinants were also detectable on the intact IgG proteins. The frequency of λVI molecules among λ-type light chains is estimated to be ~5% based on the finding that 5 of 91 λ Bence Jones proteins were of the $V_{\lambda VI}$ subgroup. Proteins of the $V_{\lambda VI}$ subgroup, in contrast to those of the other five chemically-classified λ chain subgroup, appear to be preferentially associated with the amyloid process as evidenced by the fact that all six λVI proteins were obtained from patients with amyloidosis AL and, in addition, 5 of 42 λ-type monoclonal immunoglobulins from patients with primary or myeloma-associated amyloidosis were classified by immunodiffusion analyses as having λVI-type light chains.

INTRODUCTION

Light polypeptide chains of immunoglobulins, especially their variable domain-related fragments, are the major protein constituents of the amyloid fibrillar substance found in patients with primary or multiple myeloma-associated amyloidosis (reviewed in ref. 1). This light chain form of amyloidosis, designated AL, is distinguished chemically from the protein fibril constituents of amyloid found in association with chronic inflammatory diseases or deposited in endocrine organs, heart, and brain. Certain types of light chains may be more "amyloidogenic" than others; this possibility is suggested by the observation that λ-chain-containing monoclonal immunoglobulins (complete molecules or Bence Jones proteins) are found more frequently than are κ-chain proteins among patients with primary or myeloma-associated amyloidosis. In contrast, κ-chain-type monoclonal immunoglobulins are found more commonly than are λ-chain proteins among patients with multiple myeloma or related B cell neoplasms who do not have amyloidosis (2).

Distinctive physicochemical properties of light chains are related, in part, to structural features of the V_L (3). Multiple subgroups of human λ- and κ-light chains have been defined chemically on the basis of characteristic amino acid residues within the first framework region (FR1) comprising the N-terminal 23 residues of the variable domain of the light chain (V_L).[1] For λ-chains, six variable (V) region subgroups, designated $V_{\lambda I}$, $V_{\lambda II}$, $V_{\lambda III}$, $V_{\lambda IV}$, $V_{\lambda V}$, and $V_{\lambda VI}$, have been delineated through sequence analyses of Bence Jones proteins and light chains isolated from monoclonal immunoglobulins (4). Proteins of the λVI subgroup are of special interest because two of the three prototype

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[1] *Abbreviations used in this paper:* AL, light chain form of amyloidosis; FR1, framework region 1; PTH, phenylthiohydantoin; C, constant domain; V, variable domain; V_L, light chain variable domain.

[2] The λ-chain V region subgroup λVI was originally designated λV (5).

λVI proteins[2] were found in the splenic fibrils of patients with primary amyloidosis (5, 6).

We have identified and characterized six additional λVI light chains (five Bence Jones proteins and the light chains of a monoclonal IgGλ protein) all of which were obtained from patients with primary or myeloma-associated amyloidosis. We demonstrate the isotypic nature of the $V_{\lambda VI}$ subgroup and provide further evidence that λVI proteins are most commonly found among a subset of patients with the AL(λ) form of amyloidosis.

METHODS

The diagnosis of amyloidosis AL was based on the clinical and pathological features characteristic of patients with primary or myeloma-associated forms of this disease (1). The presence of amyloid deposits was substantiated by finding (through polarizing microscopy) birefringent-staining material within Congo Red-treated tissue. The fibrillar nature of the amyloid deposits (1) was also noted in those patients from whom tissues were obtained for ultrastructural study.

Bence Jones proteins were isolated from urine specimens (7) by zone (block) electrophoresis on polyvinyl chloride (Pevikon, Kemanord, Stockholm, Sweden) and further purified by gel filtration through AcA 54 Ultrogel agarose-polyacrylamide (LKB-Produkter AB, Bromma, Sweden) columns (2.5 × 100 cm) containing a 0.15-M NaCl, 0.02-M $NaPO_4$, buffer, pH 7.2. Immunoglobulin components were also isolated from serum specimens by zone electrophoresis on polyvinyl chloride blocks. Pooled normal human γ-globulin (IgG), Cohn Fraction II, was obtained from Mann Research Laboratories, New York. Light chains were isolated from reduced and alkylated IgG by gel filtration through Bio-Gel P-100 polyacrylamide (Bio-Rad Laboratories, Richmond, CA) columns equilibrated with 1 M propionic acid. The purity and molecular weight of the isolated proteins were determined by electrophoresis in sodium dodecyl sulfate (SDS)-polyacrylamide gels containing 0.1 M β-mercaptoethanol. Amyloid fibrils were isolated from the spleen (8) and the protein extracted by gel filtration through an AcA 54 column in the presence of 5 M guanidine HCl-1 M acetic acid containing 0.1 M dithiothreitol (9).

Immunodiffusion and immunoelectrophoretic analyses were performed in 1% (wt/vol) agar gels prepared in a 0.05-M sodium barbital buffer, pH 8.6, containing 3% polyethylene glycol 6000. Antisera to γ, α, μ, δ, and ε heavy chains and to κ and λ light chains were prepared in albino New Zealand rabbits (7) by immunization with monoclonal IgG, IgA, IgM, IgD, and IgE proteins and with κ- and λ-type Bence Jones proteins. The antisera were rendered monospecific for heavy or light chain determinants by appropriate absorption.

Amino acid sequence analyses were performed (10) with a Beckman model 890C automated sequencer (Beckman Instruments, Inc., Fullerton, CA). The phenylthiohydantoin (PTH) derivatives were identified by high performance liquid chromatography using a Waters HPLC Model ALC/GPC-204 and a C18/μ-Bondapak column (Waters Instruments, Inc., Rochester, MN). A methanol:water gradient was used for elution. In some instances the PTH derivatives were hydrolyzed and analyzed with a Durrum Model D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, CA).

RESULTS

Immunological recognition of the human λ-light chain V region subgroup λVI ($V_{\lambda VI}$). An antiserum prepared against the λ-Bence Jones protein SUT was found by immunodiffusion analyses to be capable of distinguishing among heterologous λ-light chains. This antiserum, designated R394, had major specificity for the protein used for immunization and for several other λ-chains, e.g., Bence Jones protein GIO. To determine whether antiserum R394 could specifically recognize a particular V region-related λ-subgroup, we compared the reactivity of protein GIO with that of λ-light chains representative of five of the six chemically-classified V region subgroups $V_{\lambda I}$, $V_{\lambda II}$, $V_{\lambda III}$, $V_{\lambda IV}$, and $V_{\lambda V}$ (4). As shown in Fig. 1A, the λI, λII, λIII, λIV, and λV proteins formed precipitin reactions of identity, all of which were antigenically deficient to that formed by protein GIO. Absorption of antise-

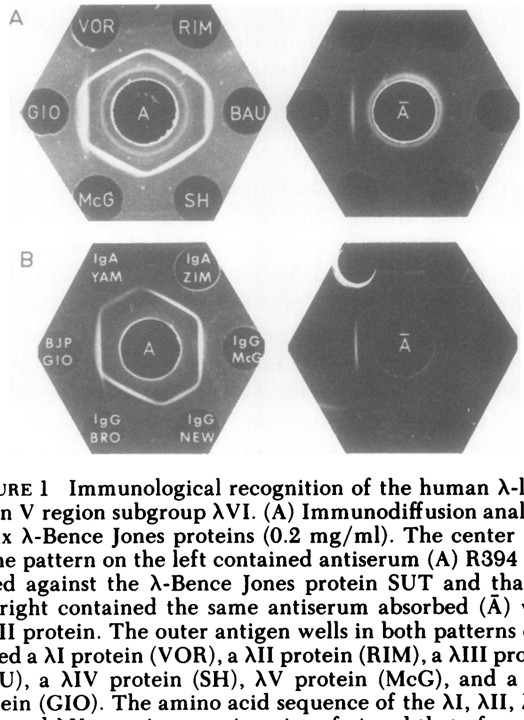

FIGURE 1 Immunological recognition of the human λ-light chain V region subgroup λVI. (A) Immunodiffusion analyses of six λ-Bence Jones proteins (0.2 mg/ml). The center well in the pattern on the left contained antiserum (A) R394 prepared against the λ-Bence Jones protein SUT and that on the right contained the same antiserum absorbed (Ā) with a λIII protein. The outer antigen wells in both patterns contained a λI protein (VOR), a λII protein (RIM), a λIII protein (BAU), a λIV protein (SH), λV protein (McG), and a λVI protein (GIO). The amino acid sequence of the λI, λII, λIII, λIV, and λV proteins are given in ref. 4 and that of protein GIO in Table I. (B) Immunodiffusion analyses of a λVI Bence Jones protein (BJP GIO) and five monoclonal λ-chain-containing immunoglobulins (0.5 mg/ml): An IgAλVI protein (IgA YAM), an IgAλII protein (IgA ZIM), an IgGλV protein (IgG McG), an IgGλI protein (IgG NEW), and an IgGλII protein (IgG BRO). Sequence data on the λI light chain NEW, the λV light chain McG, and the λVI light chain YAM are given in ref. 4; the light chain subgroups of proteins ZIM and BRO were determined immunologically. The center wells (A and Ā) contained the same antisera as indicated above.

rum R394 with any one of these five proteins rendered the antiserum specific for protein GIO.

When the absorbed antiserum, R394, was tested against a panel of 91 human Bence Jones proteins (partial or complete amino acid sequence data were available on 24 of these), precipitin reactivity was obtained with only 5 (proteins SUT, GIO, MOR, WAN, and WIN). No reactivity was evident with 6 λ-chains classified chemically as λI, 9 as λII, 6 as λIII, 1 as λIV, and 2 as λV, or with the remaining 62 non-λVI Bence Jones proteins.

The specificity of antiserum R394 was made evident through gel immunodiffusion analysis of a λVI light chain-containing monoclonal immunoglobulin, the IgAλ protein YAM (11). Protein YAM and Bence Jones protein GIO formed a precipitin reaction of identity and, as shown in Fig. 1B, both proteins could be readily distinguished from monoclonal immunoglobulins bearing λ-light chains representative of other chemically-defined V_λ subgroups. Thus, antiserum R394 apparently had unique specificity for λVI light chains.

Immunodiffusion analyses of 32 isolated λ-chain-type monoclonal immunoglobulins (18, IgG, 10 IgA, 4 IgM) and 32 serum specimens containing λ-type monoclonal immunoglobulins (22 IgG, 7 IgA, 3 IgD) revealed that antiserum R394 (absorbed to be specific for λVI proteins) had major specificity for only 2 proteins (IgGλ BUC and IgMλ DIB).

Sequence analyses of immunologically classified λ light chains. The specificity of antiserum R394 for λVI-type light chains was confirmed through determination of the amino-terminal sequence of the five λ-Bence Jones proteins SUT, GIO, MOR, WAN, and WIN and that of the IgGλ protein BUC. These sequence data are provided in Table I. Proteins SUT, GIO, MOR, WAN, AND WIN had amino acid residues within the FR1 characteristic for proteins of the $V_{\lambda VI}$ subgroup. Each of the proteins was homologous in sequence and closely resembled the sequences of the prototype λVI proteins AR, JAM, and YAM-L (4) and of the λVI proteins KIN-L (12), NIG-48 (13) and RS (14). The sequence of the first 6 residues of λ-chain BUC (data not shown) was also consonant with that of a λVI protein.

Identification of λVI light chains among the light chains of normal subjects. To determine whether λVI light chains are represented among an individual's λ-chain-bearing immunoglobulin population, we studied the reactivity of antiserum R394 against the light chains isolated from the IgG of 12 normal subjects. Antiserum R394, rendered specific for λVI antigenic determinants by appropriate absorption, was tested by comparative double diffusion analysis against each light chain preparation (concentration, 10 mg/ml) and against the λVI Bence Jones protein GIO. The presence of λVI molecules within each light chain pool was evidenced by the reactions of the light chains with the absorbed antiserum and, further, by the precipitin reaction of identity that each formed with the λVI protein GIO.

It was possible also to detect λVI light chain determinants on intact IgG molecules isolated from the serum of normal persons. Fraction II pooled γ-globulin at concentrations from 6.25 to 50 mg/ml were tested by gel immunodiffusion analyses against the specific anti-λVI antiserum R394. As illustrated in Fig. 2, precipitin reactions of identity were obtained between the reference monoclonal IgGλVI protein BUC (concentration, 1 mg/ml) and the IgG specimens at concentrations of 50 and 25 mg/ml. Comparable results were obtained with IgG isolated from individual (normal) serum specimens.

From our finding of 5 λVI Bence Jones proteins among 91 λ-Bence Jones proteins tested and on the results obtained with the survey of 32 λ-chain-containing monoclonal immunoglobulins, we estimate that ~5% of λ-light chains are of the $V_{\lambda VI}$ subgroup. Based on the percentage of IgG λ-chain-containing molecules in normal serum (~35%), the results of the immunodiffusion analyses of FRII γ-globulin and of IgG isolated from individual (normal) serum are consonant with this estimate.

Antigenic differences among λVI light chains. The anti-λVI protein SUT antiserum (antiserum R394) was used to compare the reactivity of the λVI Bence Jones proteins GIO, WAN, and WIN to that of protein SUT. Each of the heterologous proteins gave a precipitin reaction of identity with each other and with the homologous protein (Fig. 3A). In contrast, the use of an antiserum prepared against Bence Jones protein GIO (antiserum R408), showed that proteins GIO and WIN shared common antigenic determinants and were readily differentiated from proteins SUT and WAN (Fig. 3B). In other experiments (not illustrated), proteins MOR and WAN reacted in similar fashion. The differentiation of proteins GIO and WIN from proteins SUT, MOR, and WAN was also evident with an antiserum prepared against Bence Jones protein WIN.

Association between λVI light chains and amyloidosis AL. All five of our λVI Bence Jones proteins (plus the IgGλ protein BUC) were obtained from patients who had histochemically-documented amyloid and who were characterized clinically as having "primary" or myeloma-associated amyloidosis. The relationship between the V region subgroup of the Bence Jones protein and the presence or absence of amyloidosis is shown in Table II. Among the 91 Bence Jones proteins tested immunologically, 20 were obtained from patients with amyloidosis AL. Of these 20

TABLE I
Sequence Data on Human λVI Light Chains

λ-chain (Ref.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
AR (18)	Asp	Phe	Met	Leu	Thr	Gln	Pro	His	Ser	Val	Ser	Glu	Ser	Pro	Gly	Lys	Thr	Val	Thr	Phe	Ser	Cys
NIG-48 (13)	Asn	Leu	—	—	Ile	—	—	Pro	—	—	—	—	—	—	—	—	—	—	—	Met	—	—
RS (14)	Asn	—	—	—	—	Glu	—	—	—	—	—	—	—	—	—	Gln	—	—	—	Ile	—	—
YAM-L (11)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Gly	Lys	—	—	Ile	—	—
JAM (6)	—	—	—	—	—	Glu	—	—	—	—	—	—	—	—	—	—						
KIN-L (12)	Asn	—	—	—	Leu	—	—	—	—	—	—											
SUT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Ile	Ile	—	—
MOR	Asn	Leu	—	—	—	—	—	—	—	Leu	—	Asp	—	—	—	—	—	Ile	Ile	Ile	—	—
GIO	Asn	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Ile	—	—
WAN	Asn	—	Ile	—	—	—	—	(—)	—	—	—	—	—	—	—	—	—	—	—	Ile	—	—
WIN	Asn	—	—	—	—	—	—	(—)	—	—	—	—	—	—	—	—	—	—	—	(Ile)		

λ-chain (Ref.)	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
AR (18)	Thr	Gly	Ser	Gly	Gly	Ser	Ile	Ala	Asp	Ser	Phe	Val	Gln	Trp	Tyr	Gln	Gln	Arg	Pro	Gly	Ser
NIG-48 (13)	—	Arg	Thr	Ser	Asp	—	—	—	Ser	Asn	Tyr	—	—	—	—	Arg	—	—	—	—	
RS (14)	—	—	—	—	Asp	—															
SUT	—	Arg	—	Asp	—	Thr	—	—	Gly	Tyr	Tyr	—	—	—	—	—	—	—	—	—	Arg
MOR	—	Ala	Asn	—	—	Asn	—	Gly	Ser	His	Pro	—	(His)	—	—	Lys	?	?	—	Asp	—
GIO	—																				

The residues in the λ chains that are identical in corresponding positions to those of protein AR are indicated by a solid line; ?, residue not identified; ( ), residue tentatively identified.

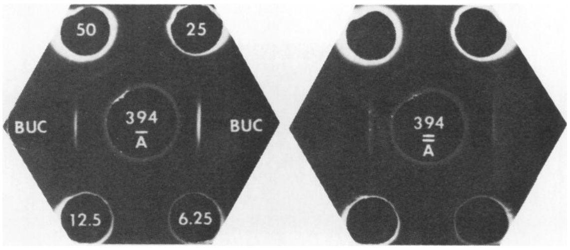

FIGURE 2 Immunological detection of λVI light chain-containing IgG in normal serum. Immunodiffusion analyses of pooled normal human γ-globulin (Cohn fraction II) and the monoclonal IgGλVI protein BUC. The center well in the pattern on the left contained an antiserum prepared against the λVI Bence Jones protein SUT and absorbed with cord serum (R394 Ā). The center well in the pattern on the right contained the same antiserum additionally absorbed with a λI Bence Jones protein (R394 Ā). The outer antigen wells in both patterns contained Cohn fraction II γ-globulin at the indicated concentrations of 50, 25, 12.5, and 6.25 mg/ml; the concentration of protein BUC was 1 mg/ml.

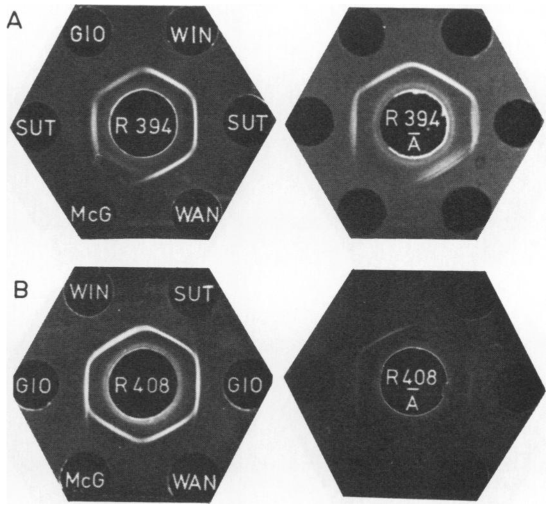

FIGURE 3 Antigenic heterogeneity of λVI light chains. Immunodiffusion analysis of λVI Bence Jones proteins SUT, GIO, WIN, and WAN and the λV Bence Jones protein McG. (A) The center well in the pattern on the left contained an antiserum (R394) prepared against protein SUT and that on the right contained the same antiserum absorbed (R394 Ā) with a λI protein. (B) The center well in the pattern on the left contained an antiserum (R408) prepared against protein GIO and that on the right the same antiserum absorbed (R408 Ā) with a λI protein.

proteins, 5 were of the $V_{\lambda VI}$ subgroup, and the remaining 15 were associated with the $V_{\lambda II}$, $V_{\lambda III}$, and $V_{\lambda V}$ subgroups.

The association of λVI-type Bence Jones proteins in patients with amyloidosis AL(λ) was evidenced further through immunodiffusion analyses of λ-chain-containing monoclonal Igs obtained from patients with documented primary or myeloma-associated amyloidosis. Antiserum R394, rendered specific for λVI antigenic determinants, was used to determine the reactivity of the monoclonal proteins in the serum specimens (furnished by Dr. Robert A. Kyle, Mayo Clinic, Rochester, MN) from 42 such patients.

As shown in Fig. 4, precipitin reactions of identity were evident by immunodiffusion analyses between the reference IgGλVI protein BUC and the immunoglobulin from certain patients (patients 20 and 21). 5 of the 42 serum specimens containing λ-type monoclonal immunoglobulins (19 IgG, 7 IgA, 2 IgM, 1 IgD, and 13 Bence Jones proteins) had protein reacting with the absorbed antiserum R394. In each case, a precipitin reaction of identity was obtained between the serum component and protein BUC. Immunoelectrophoretic analyses (performed with monospecific anti-heavy chain and anti-light chain antisera and with the specific anti-λVI antiserum R394) revealed that the precipitin reactions formed by the monoclonal Igs (two IgG, one IgA, and two Bence Jones proteins) conformed exactly to those obtained with antiserum R394.

TABLE II
Relation of λ-Bence Jones Proteins and Amyloidosis AL(λ)

		V_λ region subgroups						
Patient classification	Number	$V_{\lambda I}$	$V_{\lambda II}$	$V_{\lambda III}$	$V_{\lambda IV}$	$V_{\lambda V}$	$V_{\lambda VI}$	Non-$V_{\lambda VI}$*
Amyloid	20	0	7	3	0	1	5	4
Nonamyloid‡								
N	26	9	7	8	0	0	0	2
or								
U	45	7	12	11	1	1	0	13
Total	91	16	26	22	1	2	5	19

* No reaction when tested against the specific anti-λVI antiserum R394.
‡ N, no amyloid evident clinically and/or histologically; U, unknown.

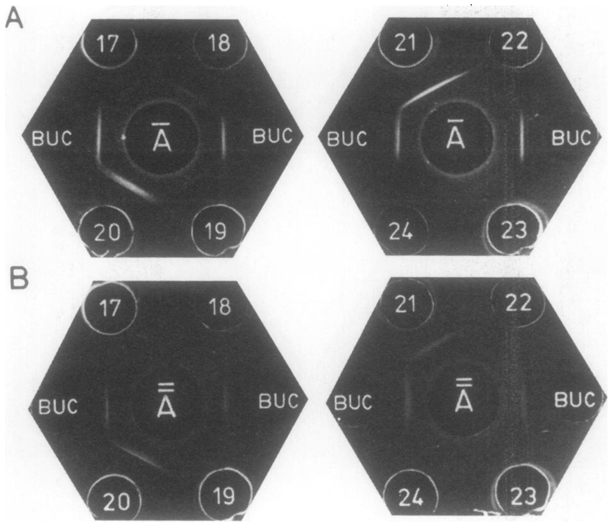

FIGURE 4 Immunodiffusion analyses of serum specimens from eight patients with primary or myeloma-associated amyloidosis AL(λ) and of the monoclonal IgG λVI protein BUC obtained from a patient with amyloidosis AL(λ). The wells marked 17, 18, 19, 20, 21, 22, 23, and 24 contained serum specimens from patients with monoclonal IgMλ, BJPλ, IgGλ, IgAλ, BJPλ, IgMλ, IgDλ, and IgGλ proteins, respectively. (A) The center wells contained Antiserum R394 absorbed (Ā) with cord serum. (B) The center wells contained the same antiserum additionally absorbed (A̿) with a serum containing a monoclonal IgAλ protein. The test serum specimens were diluted with 0.15 M NaCl so that the final concentration of the monoclonal protein was ~1 to 2 mg/ml; the concentration of protein BUC was 1 mg/ml.

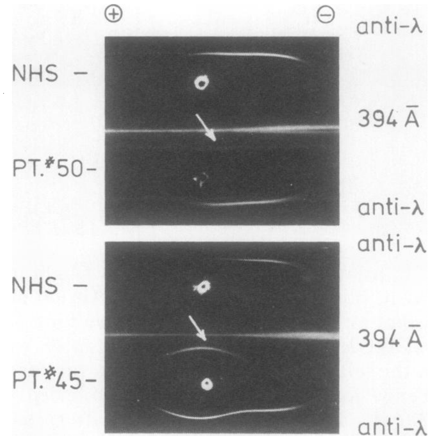

FIGURE 5 Immunological detection of λVI light chain-containing immunoglobulins in patients with primary or myeloma-associated amyloidosis AL(λ). Immunoelectrophoretic analyses of serum from a normal subject (NHS) and from two patients with amyloidosis AL(λ) (PT 50 and PT 45). The upper and lower antiserum troughs contained an anti-λ chain antiserum (anti-λ) and the middle trough contained antiserum R394 absorbed (394 Ā) to be specific for λVI light chains. The arrows indicate the λVI light chain-containing immunoglobulin in each patient's serum.

Serum specimens (also furnished by Dr. Kyle) from seven additional patients with amyloidosis AL had no evident monoclonal immunoglobulin when examined by cellulose acetate electrophoresis or by immunoelectrophoresis in which antisera specific for γ, α, μ, δ, and ϵ heavy chains and for κ and λ light chains were used. However, immunodiffusion analyses revealed that two of the seven specimens contained a component that reacted with the absorbed antiserum R394 and also formed a precipitin reaction of identity with protein BUC. In each case, a "monodisperse" IgGλVI serum protein was readily identified by immunoelectrophoretic analyses using the monospecific anti-IgG and anti-λVI antiserum R394 (Fig. 5). The sensitivity of this method would not permit the detection of IgGλVI molecules in the specimens of normal serum.

Relation between λVI amyloid and urinary light chain proteins. In the case of our patient GIO, both the excreted Bence Jones protein and the protein extracted from splenic amyloid fibrils were available for study. The two proteins could be readily distinguished on the basis of molecular weight and antigenic determinants. As determined by gel filtration and SDS polyacrylamide gel electrophoresis, the spleen-derived protein was ~18,000 vs. ~22,500 daltons for the monomeric form of the native Bence Jones protein. The lower molecular weight of the amyloid fibril protein was attributed to the absence of a portion of the constant (C) region of the light chain. In contrast to the Bence Jones protein, the fibril protein lacked common λ-chain antigenic determinants including the "hidden" C region determinant (15). The V regions of the amyloid fibril protein GIO and Bence Jones protein GIO appeared to be identical based on N-terminal sequence and immunological analyses. Both proteins possessed the same V region idiotypic and isotypic antigenic determinants as judged from their precipitin reactions of identity with an antiserum prepared against the native Bence Jones protein GIO and with the specific anti-λVI antiserum R394, respectively.

DISCUSSION

The V region subgroup of human λ-light chains, designated $V_{\lambda VI}$, was recognized initially through sequence analyses of protein extracted from the splenic fibrils of two patients, AR (5) and JAM (6), who had primary amyloidosis. Four additional λVI proteins have since been identified from sequence analyses of the light chains isolated from the IgAλ protein YAM (11), the IgGλ protein KIN (12), the Bence Jones protein NIG-48 (13), and the protein isolated from amyloid fibrils of patient RS (14). Variable region subgroups of human κ- and λ-light chains have been also identified immunochemically (3, 16). Concordance between the structural and immunological classification of λ-chains has been demonstrated for the chemically-defined $V_{\lambda I}$, $V_{\lambda II}$, and $V_{\lambda III}$ subgroups.[3] Amyloid fibrillar proteins AR and RS were shown to have similar V region antigenic determinants when tested with antisera prepared against the alkali-denatured protein AR (14).

We found that an antiserum prepared against the λ-Bence Jones protein SUT from a patient with myeloma-associated amyloidosis had unique specificity for antigenic determinants associated with the V region of λVI light chains. Through comparative immunological studies of λ-Bence Jones proteins and λ-type monoclonal immunoglobulins, five additional proteins that shared V region determinants with protein SUT were identified. Subsequently, all six proteins were shown to have N-terminal sequences characteristic for the $V_{\lambda VI}$ subgroup.

We estimate the proportion of IgG molecules bearing λVI light chains to be ~5% based on the identification of 5 λVI proteins among 91 λ-Bence Jones proteins tested and on the results of immunodiffusion analyses of IgG in normal human serum. Our demonstration of λVI protein among the light chains isolated from the IgG of 12 normal individuals (as well as within the IgG pool) indicates the isotypic nature of the $V_{\lambda VI}$ subgroup, as has been shown for other V region light chain subgroups (3). We presume the $V_{\lambda VI}$ subgroup represents a distinct V segment gene product, based on nucleic acid hybridization probes of human DNA that have indicated light chain V region subgroups (e.g., $V_{\kappa I}$ and $V_{\kappa III}$) are part of the germ-line gene pool (17). Two populations of λVI proteins were distinguished with our anti-λVI Bence Jones protein antisera. Intrasubgroup V region antigenic heterogeneity has been well-documented among κ-(18) and λ-light chains.[3] Whether these "sub-subgroups" are products of separate germ-line genes or, alternatively, result from somatic mutation remains to be determined.

In those cases where clinical and histological data are available, λVI light chains appear to be found preferentially in patients with amyloidosis AL. In addition to the two prototype λVI proteins AR (5) and JAM (6), Protein RS (13) was isolated from splenic amyloid fibrils.[4] All of our λVI Bence Jones proteins—SUT, GIO, MOR, WAN, WIN—and the IgGλ protein BUC were obtained from patients who also had primary or myeloma-associated amyloidosis. Further, our analyses by gel immunodiffusion of λ-chain-containing monoclonal immunoglobulins from 42 patients with amyloidosis AL revealed that 5 were of the $V_{\lambda VI}$ subgroup—a twofold higher incidence than expected based on the frequency of occurrence for this V region isotype among human λ-chains. In addition, a "monodisperse" IgGλVI protein was detected in the serum of two of seven additional such patients.

Based on the data from our six patients and from the seven at the Mayo Clinic (Dr. R. A. Kyle, personal communication)—all of whom had λVI-type monoclonal proteins—no clinical or pathologic features of patients with amyloidosis AL(λVI) were apparent that would distinguish them from patients with amyloidosis AL associated with other subgroups of λ-light chains (or of κ-chains).

The relationship between λVI light chains and the amyloid process is presently unknown. These proteins may possess distinctive V region chemical features that permit them to assume the twisted β-pleated sheet characteristic for amyloid fibrils (1). Alternatively, the structure of these proteins may render them amyloidogenic due to their unusual susceptibility to proteolysis. With rare exceptions (19), the majority of proteins extracted from amyloid fibrils of patients with the AL form of amyloidosis consist of V_L-related fragments or of light chain protein containing the V_L plus a portion of the C region (1). Each of the three λVI proteins (AR, JAM, and RS) isolated from splenic amyloid fibrils was characterized by an amino acid composition and molecular weight indicative of a protein of lower molecular weight than that of the monomeric unit of an intact (complete) light chain. Protein AR consisted of an ~154 residue polypeptide containing the entire V region and the first 42 residues of the C region (20). For patients AR and JAM, no urinary Bence Jones protein or light chain-containing monoclonal immunoglobulin was available for comparative analysis with the protein isolated from the amyloid fibrils. The molecular weight and antigenic determinants of the amyloid fibril protein from our patient GIO also indicate that this component is comparable in size to that of protein AR and most likely represents a fragment of Bence Jones protein GIO that contains the entire V region plus a portion of the C region. The presence of an intact λVI light chain and a smaller component corresponding to the amyloid fibril protein was not detected in urine specimens from patient GIO, in contrast to that noted for patient RS (14). Light chain fragments were not evident in urine specimens from our patients SUT and MOR. The heterogeneity in the C-terminal residue of the polypeptide fragments of amyloid protein AR (20) is indicative of its catabolic (proteolytic) origin from an intact light chain rather than an aberrant product of synthesis. Leukocyte-derived neutral proteases are capable of

[3] Solomon, A. Manuscript in preparation.

[4] Patients YAM (11) and NIG-48 (13) had multiple myeloma; patient KIN (12) had idiopathic cryoglobulinemia; and patient DIB had Waldenström's macroglobulinemia. No histologic data were available to establish the presence of amyloidosis in these patients.

cleaving light chains into V_L-related fragments (21), thus implying a functional role for the reticuloendothelial system in the pathogenesis of amyloidosis (1, 22, 23).

Because of the association of λVI light chains with amyloidosis AL, the availability of a rapid and sensitive technique to recognize such proteins has considerable clinical import. Indeed, based on our initial detection immunologically of λVI proteins in urine specimens from two patients with nephrotic syndrome, the diagnosis of amyloidosis AL was confirmed histologically from specimens obtained by renal biopsy. Studies are in progress to characterize in detail λVI light chains and to define their role in the pathogenesis of amyloidosis AL.

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REFERENCES

1. Glenner, G. G. 1980. Amyloid deposits and amyloidosis. The β-fibrilloses. *N. Engl. J. Med.* **302:** 1283–1292; 1333–1343.
2. Glenner, G. G., W. D. Terry, and C. Isersky. 1973. Amyloidosis: Its nature and pathogenesis. *Semin. Hematol.* **10:** 65–86.
3. Solomon, A. 1976. Bence-Jones proteins and light chains of immunoglobulins. *N. Engl. J. Med.* **294:** 17–23; 91–98.
4. Kabat, E. A., T. T. Wu, and H. Biolofsky. 1979. Sequences of Immunoglobulin Chains. Tabulations and Analysis of Amino Acid Sequences of Precursors, V-regions, C-regions, J-chain, and β_2-microglobulins. U. S. Department of Health, Education and Welfare, Washington, D. C.
5. Sletten K., G. Husby, and J. B. Natvig. 1974. N-terminal amino acid sequence of amyloid fibril protein AR, prototype of a new λ-variable subgroup $V_{\lambda V}$. *Scand. J. Immunol.* **3:** 833–836.
6. Skinner, M., M. D. Benson, and A. S. Cohen. 1975. Amyloid fibril protein related to immunoglobulin λ-chains. *J. Immunol.* **114:** 1433–1435.
7. Solomon, A., and C. L. McLaughlin. 1969. Bence-Jones proteins and light chains of immunoglobulins. I. Formation and characterization of amino-terminal (variant) and carboxyl-terminal (constant) halves. *J. Biol. Chem.* **244:** 3393–3404.
8. Pras, M., M. Schubert, D. Zucker-Franklin, A. Rimon, and E. C. Franklin. 1968. The characterization of soluble amyloid prepared in water. *J. Clin. Invest.* **47:** 924–933.
9. Glenner, G. G., M. Harada, and C. Isersky. 1972. The purification of amyloid fibril proteins. *Prep. Biochem.* **2:** 39–51.
10. Michealsen, T. E., B. Frangione, and E. C. Franklin. 1977. Primary structure of the "hinge" region of human IgG3. Probable quadruplication of a 15-amino acid residue basic unit. *J. Biol. Chem.* **252:** 883–889.
11. Wang, A. C., H. H. Fudenberg, I. Y. Wang, and A. Watanabe. 1976. Chemical and genetic characterization of a monoclonal IgA (λ) protein with bone cryo- and pyro-precipitability. *Scand. J. Immunol.* **5:** 311–316.
12. Abraham, G. N., D. N. Podell, R. Wistar, Jr., S. L. Johnston, and E. H. Welch. 1979. Immunological and structural properties of human monoclonal IgG cryoglobulins. *Clin. Exp. Immunol.* **36:** 63–70.
13. Takahashi, N., T. Takayasu, T. Isobe, T. Shinoda, T. Okuyama, and A. Shimizu. 1979. Comparative study on the structure of the light chains of human immunoglobulins. II. Assignment of a new subgroup. *J. Biochem.* **86:** 1523–1535.
14. Natvig, J. B., P. Westermark, K. Sletten, G. Husby, and T. Michaelsen. 1981. Further structural and antigenic studies of light-chain amyloid proteins. *Scand. J. Immunol.* **14:** 89–94.
15. Solomon, A. 1976. Bence Jones proteins and light chains of immunoglobulins. XIV. Conformational dependency and molecular localization of the kappa (κ) and lambda (λ) antigenic determinants. *Scand. J. Immunol.* **5:** 685–695.
16. Tischendorf, F. W., M. M. Tischendorf, and E. F. Osserman. 1970. Subgroup-specific antigenic marker on immunoglobulin λ-chains: identification of three subtypes of the variable region. *J. Immunol.* **105:** 1033–1035.
17. Bentley, D. L., and T. H. Rabbitts. 1981. Human V_κ immunoglobulin gene number: Implication for the origin of antibody diversity. *Cell.* **24:** 613–623.
18. McLaughlin, C. L., and A. Solomon. 1972. Bence-Jones proteins and light chains of immunoglobulins. VII. Localization of antigenic sites responsible for immunochemical heterogeneity of κ-chains. *J. Biol. Chem.* **247:** 5017–5025.
19. Terry, W. D., D. L. Page, S. Kimura, T. Isobe, E. F. Osserman, and G. G. Glenner. 1973. Structural identity of Bence Jones and amyloid fibril proteins in a patient with plasma cell dyscrasia and amyloidosis. *J. Clin. Invest.* **52:** 1276–1281.
20. Sletten, K., J. B. Natvig, G. Husby, and J. Juul. 1981. The complete amino acid sequence of a prototype immunoglobulin-λ light-chain-type amyloid-fibril protein AR. *Biochem. J.* **195:** 561–572.
21. Solomon, A., W. Schmidt, and K. Havemann. 1976. Bence Jones proteins and light chains of immunoglobulins. XIII. Effect of elastase-like and chymotrypsin-like neutral proteases derived from human granulocytes on Bence Jones proteins. *J. Immunol.* **117:** 1010–1014.
22. Rosenthal, C. J., and E. C. Franklin. 1977. Amyloidosis and amyloid proteins. *In* Recent Advances in Clinical Immunology. R. A. Thompson, editor. Churchill Livingstone, Inc., New York. **1:** 41–76.
23. Cohen, A. S., and E. S. Cathcart. 1980. Amyloidosis. *In* Contemporary Hematology/Oncology. J. LoBue, A. S. Gordon, R. Silber, and F. M. Muggia, editors. Plenum Publishing Co., New York. **1:** 243–276.