SEQUENCE SIMILARITIES AMONG κIIIb CHAINS OF MONOCLONAL HUMAN IgMκ AUTOANTIBODIES

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Considerable insight into the structural and genetic bases of antibody specificity has come from studies of the homogeneous immunoglobulin products of monoclonal B cell disorders, especially those proteins of defined antibody specificity. The seminal finding of Kunkel and co-workers (1, 2) that human monoclonal autoantibodies express distinctive isotypic and cross-idiotypic determinants and have common structural features provided the initial evidence for a genetically restricted immune response in man to self antigens. Their studies demonstrated that the light (L)[1] chains of monoclonal IgMκ cold agglutinins and of IgMκ anti-IgG autoantibodies, i.e., rheumatoid factors (RFs), were predominantly members of the V region subgroup $V_{\kappa III}$ (2–4); the similarity among these proteins was also evidenced by the finding that 10 of the 16 κIII chains from the IgMκ RFs had properties associated with the serologically and chemically defined κIIIb subsubgroup (5, 6). Sequence analyses of IgM RFs also demonstrated extensive V region similarities among proteins expressing cross-reactive idiotypic determinants (7, 8).

Our immunochemical and biochemical analyses of 10 monoclonal IgMκ proteins isolated from IgM-IgG cryoprecipitable immune complexes (9, 10) confirmed the observation of Kunkel et al. (2) by demonstrating that their light chains were κIIIb (10). Further evidence for the preferential association of κIIIb light chains with monoclonal IgMκ autoantibodies was obtained by our finding that the light chains of three IgMκ anti-low density lipoprotein (LDL) antibodies were also members of the κIIIb sub-subgroup. Partial amino acid sequence data on all 13 κ chains revealed marked similarities in primary structure (10).

To establish the extent of homology among the κ chains of IgMκ autoantibod-

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[1] *Abbreviations used in this paper:* C, constant region; CDR, complementarity-determining region; FR, framework region; HAc, acetic acid; HDL, high density lipoprotein, HPLC, high performance liquid chromatography; J, joining gene segment; L, light chain; LDL, low-density lipoprotein; PBS, phosphate-buffered saline; PTH, phenylthiohydantoin; RF, rheumatoid factor; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; V, variable region; VLDL, very low density lipoprotein.

ies, we have determined the complete light chain variable region amino acid sequence from four such light chains: two were from IgMκ RFs and one was from an IgMκ anti-LDL antibody; the fourth κ chain sequenced was isolated from an IgMκ protein that bound specifically intermediate filaments. Our finding that the amino acid sequence of that portion of the V_L region encoded by the V gene segment (positions 1–95) of all four κ light chains is virtually identical provides further evidence that the $V_{\kappa IIIb}$ germ line gene is selected preferentially in the human IgM autoimmune response.

Materials and Methods

Isolation of Monoclonal IgMκ Proteins. Plasma was obtained by plasmapheresis of four patients: Two (GAR and GOT) had mixed cryoglobulinemia, patient SON had lymphocytic lymphoma and cryoglobulinemia, and patient PIE had Waldenström's macroglobulinemia. For proteins SON, GAR, and GOT, maximum cryoglobulin precipitation was obtained by maintaining the serum at 4°C for 48 h, after which the cryoglobulins were isolated by centrifugation at 4°C. The cryoprecipitates were washed with cold phosphate-buffered saline (PBS), pH 7.0, as previously described (11). Protein PIE was obtained as a euglobulin (12). The cryoprecipitates SON, GAR, and GOT were dissolved in 0.1 M acetic acid (HAc) and gel filtered at room temperature through a G-200 Sephadex column (140 × 4 cm) equilibrated in 0.1 M HAc. Protein PIE was dissolved in PBS, gel filtered at room temperature through a G-200 Sephadex column (150 × 4 cm) in PBS, and reprecipitated as a euglobulin. The IgM-containing fractions, identified by immunodiffusion analyses, were pooled, and their purity was determined via electrophoresis on 0.1% sodium dodecyl sulfate (SDS)-10% polyacrylamide gels and by immunodiffusion analyses with monospecific anti-heavy chain and anti-light chain antisera. The IgM proteins were assayed for anti-IgG activity via the latex fixation test and for anti-LDL activity via immunodiffusion analysis with purified LDL, very low density lipoprotein (VLDL), high density lipoprotein (HDL), and with apolipoprotein B. The binding of IgMκ protein PIE to intermediate filaments was demonstrated immunohistologically by Dr. Max D. Cooper who generously supplied plasma from this patient for our studies.

Isolation and Characterization of Light Chains from Monoclonal IgM Proteins. Light chains were obtained from partially or completely reduced IgM proteins by dissolving the lyophilized preparations in an "incomplete" reduction buffer (27 mM Tris-HCl, pH 8.2, containing 10 mM dithiothreitol) or in a "complete" reduction buffer (6 M guanidine-27 mM Tris-HCl, pH 8.2, containing 20 mM dithiothreitol), respectively. The solutions were maintained for 1 h at 37°C, and the proteins alkylated by the addition of iodo (^{14}C) acetic acid (0.7 mCi/mmol; New England Nuclear, Boston, MA) to final concentrations of 22 mM and 44 mM, respectively. After 1 h at 37°C, the protein solutions were dialyzed for three days against distilled water and lyophilized. The light chains were isolated by gel filtration of completely reduced and alkylated IgM proteins through a G-100 Sephadex column (165 × 3 cm) in 1 M HAc-5M guanidine and repurified by high performance liquid chromatography (HPLC). Their purity was established via SDS-polyacrylamide gel electrophoresis. The κ light chains obtained from partially reduced IgM proteins were analyzed via immunodiffusion analyses with antisera rendered specific for each of the four Vκ region subgroups, $V_{\kappa I}$, $V_{\kappa II}$, $V_{\kappa III}$, and $V_{\kappa IV}$ (3), and with antisera that recognized antigenic determinants associated with proteins of the κIIIb sub-subgroup (5, 6).

Enzymatic Digestion and Purification of Peptides. Completely reduced and (^{14}C) alkylated light chains were dissolved (10 mg/ml) in 0.2 M ammonium bicarbonate, pH 8.0, and the proteins digested with bovine TPCK-treated trypsin (Sigma Chemical) at 1:100 (wt/wt) enzyme/protein ratio. The digestion was performed at 37°C and after 20 min proteolysis was terminated by adding distilled water and freeze drying the sample. The tryptic peptides were separated at room temperature by HPLC (Waters Assoc., Milford, MA), using a reverse phase support medium, μBondapak C_{18}, 7.8 mm × 30 cm (13). The peptides were eluted at a flow rate of 2 ml/min using a 60-min–linear gradient generated

by mixing 0.05% trifluoroacetic acid (TFA) with 0.05% TFA/acetonitrile 1:2 (vol/vol). The column effluent was monitored by absorbance at 210 nm, and the radioactivity determined by scintillation counting (Beckman LS-250). When necessary, the peptides were repurified on a μBondapak C_{18} column 3.9 mm × 30 cm; the optimum times and TFA-TFA/acetonitrile linear gradients used were determined by the elution profiles obtained during the initial separation procedures.

Amino Acid Analysis. The tryptic peptides were hydrolyzed under reduced pressure in 0.2 ml of 6 M HCl for 20 h at 110°C (40 μl of a 1% aqueous solution of phenol were added to prevent tyrosine degradation) and their amino acid composition determined on a Durrum D-500 automatic amino acid analyzer (Dionex, Sunnyvale, CA) (13).

Amino Acid Sequence Analysis. Automated amino acid sequence analyses were performed on a Beckman 890C sequencer using a 0.1 M Quadrol program. For small peptides, polybrene was added to the cup (14). Phenylthiohydantoin (PTH) amino acids were identified by HPLC with the use of a Waters μBondapak C_{18} column, 3.9 mm × 30 cm, using a linear gradient generated by mixing solvent A, composed of 14% methanol—0.2% acetic acid (brought to pH 4.5 with triethylamine), and solvent B, of 90% methanol—0.025% acetic acid, and by automated amino acid analysis, after back-hydrolysis of the derivatives in 0.2 ml of 6 M HCl—5 μl 5% $SnCl_2$ under reduced pressure for 4 h at 150°C. Cysteine was also detected by counting the radioactivity of PTH (^{14}C) carboxymethylcysteine in a liquid scintillation counter.

Results

The completely reduced and (^{14}C) alkylated light chains were subjected to automated amino acid sequence analyses from which were determined the first 62 residues of proteins SON, GAR, and GOT, and the first 63 of protein PIE (Fig. 1). The amino terminal sequence of all four proteins was identical except for an arginyl residue at position 29 in κ chain GOT and an asparaginyl residue at position 60 for κ chain SON.

Completely reduced and (^{14}C) alkylated light chains from each protein were digested with trypsin and the resultant peptides purified by HPLC. 20 tryptic peptides, T1–T20, were obtained from each protein; their amino acid compositions are shown in Tables I and II. The κ chain V region residues (positions 1–108) were contained in peptides T1–T9; their order was readily ascertained by automated sequence analysis of each peptide separated by HPLC (Fig. 2). Tryptic peptides, T1–T5, included the first 61 residues and, in all four proteins, were identical in composition and in sequence except for the two differences noted above. As expected, the presence of the trypsin-sensitive arginyl residue at position 29 in protein GOT yielded, for T3, two peptides T3a and T3b, which contained residues from positions 25–29 and 30–45, respectively. Peptide T6 contained the residues located from positions 62–77; these were identical in all four proteins. The elution profiles of peptide T7 from proteins SON, PIE, and GAR (and peptide T7a from protein GOT) differed, ranging from 58% to 62% of the TFA-TFA/acetonitrile linear gradient (Fig. 2). For κ chain GOT, the presence of a trypsin-sensitive arginyl residue at position 96 resulted in two T7 peptides, T7a and T7b. The sequences of these peptides, which contained residues located from positions 78–103, including the radiolabeled cysteine at position 88, was similar for all three proteins and for protein GOT except at position 96, where each of the four proteins had a different residue, and at position 100, where κ chain SON differed from proteins PIE, GAR, and GOT by having a glycyl instead of a glutamyl residue. Protein SON was also unique

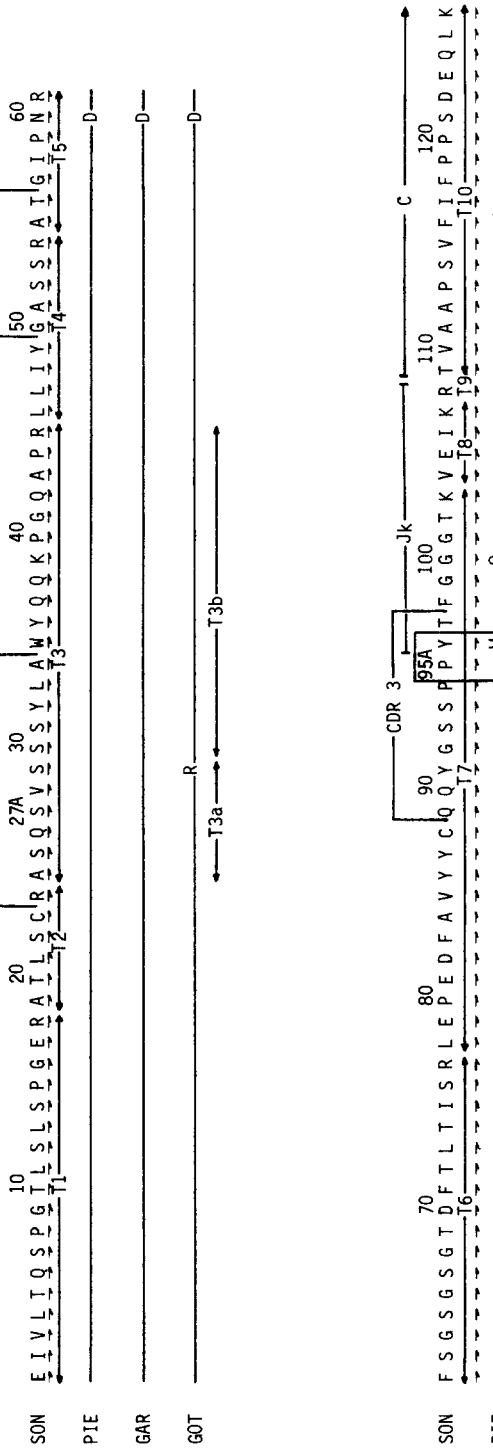

FIGURE 1. Complete V region and partial C region amino acid sequence of light chains from four monoclonal IgMκ autoantibodies, SON, PIE, GAR, and GOT. The positions are numbered according to reference 3. The solid lines indicate residues in κ chains, PIE, GAR, and GOT identical to those in protein SON. T, tryptic peptides (Tables I and II); → indicates automated determined sequence. The portions of the light chains encoded by the V and J gene segments are so indicated; the site of combinatorial joining of these segments is encompassed by a box that includes the residues at positions 95A and 96. CDR, complementarity-determining region; FR, framework region; C, constant region. The amino acids are designated by the one-letter code: A, Ala; R, Arg; N, Asn; D, Asp; C, Cys; Q, Gln; E, Glu; G, Gly; H, His; I, Ile; L, Leu; K, Lys; F, Phe; P, Pro; S, Ser; T, Thr; W, Trp, Y, Tyr; V, Val.

TABLE I

Amino Acid Composition of Tryptic (T) Peptides Obtained from the V_L Regions of Proteins SON, PIE, GAR, and GOT (Residues/mol)

Peptide	T1	T2	T3	T3a	T3b	T4	T5	T6	T7	T7	T7	T7a	T7b	T8′	T8″	T9
Protein	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR	GOT	GOT	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON	PIE	GAR	GOT	GOT	SON-PIE	GAR-GOT	SON-PIE GAR-GOT
Position	1–18	19–24	25–45	25–29	30–45	46–54	55–61	62–77	78–103	78–103	78–103	78–96	97–103	104–107	104–107	108
Cys*	—	0.72	—	—	—	—	—	—	0.63	0.77	0.74	0.70	—	—	—	—
Asp	—	—	—	—	—	—	1.09	1.18	1.18	1.10	1.08	1.12	—	—	—	—
Thr	1.88	0.87	—	—	—	—	0.93	2.83	2.00	1.07	1.97	—	1.99	—	—	—
Ser	2.66	0.81	4.88	1.81	1.93	1.81	—	3.81	1.94	1.80	2.01	2.04	—	—	—	—
Glu	3.12	—	4.05	1.06	3.18	—	—	—	4.05	4.95	5.03	3.92	1.09	0.96	0.98	—
Pro	2.02	—	2.02	—	2.13	—	0.90	—	3.02	1.96	1.89	1.98	—	—	—	—
Gly	2.09	—	1.18	—	0.98	1.18	1.12	3.09	3.92	2.80	2.98	1.18	1.90	—	—	—
Ala	—	1.07	2.96	1.05	2.03	1.09	0.95	—	1.21	1.06	1.13	1.09	—	—	—	—
Val	0.98	—	1.15	1.18	—	—	—	—	1.18	1.08	1.03	1.03	—	1.23	—	—
Ile	0.93	—	—	—	—	0.89	0.85	0.97	—	—	—	—	—	0.77	0.93	—
Leu	2.97	1.18	1.09	—	1.07	1.90	—	1.08	1.06	1.03	1.12	1.01	—	—	0.97	—
Tyr	—	—	1.79	—	2.10	1.03	—	—	3.86	2.77	3.98	2.98	—	—	—	—
Phe	—	—	—	—	—	—	—	1.87	1.86	1.83	1.93	0.93	0.95	—	—	—
His	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lys	—	—	0.98	—	1.08	—	—	—	1.21	1.00	1.05	—	1.06	1.03	1.10	—
Arg	1.09	1.11	1.20	1.08	1.03	1.03	1.04	1.13	—	—	—	1.02	—	—	—	1.00
Trp‡	—	—	1.00	—	1.00	—	—	—	—	1.00	—	—	—	—	—	—

* Determined as carboxymethylcysteine.

‡ Determined by amino acid sequence.

TABLE II

Amino Acid Composition of Tryptic (T) Peptides Obtained from the C_L Regions of Proteins SON, PIE, GAR, and GOT (residues/mol)

Peptide	T10	T11	T12	T13	T14	T15	T16	T17	T18′	T18″	T19	T20
Protein	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR GOT	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR GOT	SON	PIE GAR-GOT	SON-PIE GAR-GOT	SON-PIE GAR-GOT
Position	109–126	127–142	143–145	146–149	150–169	170–183	184–188	189–190	191–207	191–207	208–211	212–214
Cys*	—	0.56	—	—	—	—	—	—	0.67	0.79	—	0.73
Asp	1.14	2.04	—	—	4.10	1.02	0.98	—	—	—	1.02	—
Thr	1.03	1.05	—	—	0.94	2.92	—	—	1.93	1.71	—	—
Ser	2.00	2.02	—	—	3.92	4.81	—	—	1.98	2.08	0.96	—
Glu	2.21	—	1.02	0.98	5.03	—	1.13	—	2.07	2.13	—	1.19
Pro	3.01	1.10	—	—	—	—	—	—	1.15	1.07	—	—
Gly	—	1.36	—	—	1.20	—	—	—	1.18	1.19	—	1.23
Ala	1.90	1.14	1.12	—	1.24	—	1.05	—	1.28	1.21	—	—
Val	1.95	1.72	—	1.06	2.17	—	—	—	2.05	2.89	—	—
Ile	0.89	—	—	—	—	—	—	—	—	—	—	—
Leu	1.10	1.96	—	—	1.22	3.23	—	—	1.89	1.06	—	—
Tyr	—	1.11	—	—	—	1.02	—	—	0.86	0.99	—	—
Phe	1.85	1.02	—	—	—	—	0.84	—	—	—	0.95	—
His	—	—	—	—	—	—	—	0.89	1.12	1.03	—	—
Lys	1.14	—	1.08	0.95	1.21	1.17	0.98	1.10	1.26	1.36	—	—
Arg	—	1.00	—	—	—	—	—	—	—	—	1.05	—
Trp‡	—	—	—	1.00	—	—	—	—	—	—	—	—

* Determined as carboxymethylcysteine.

‡ Determined by amino acid sequence.

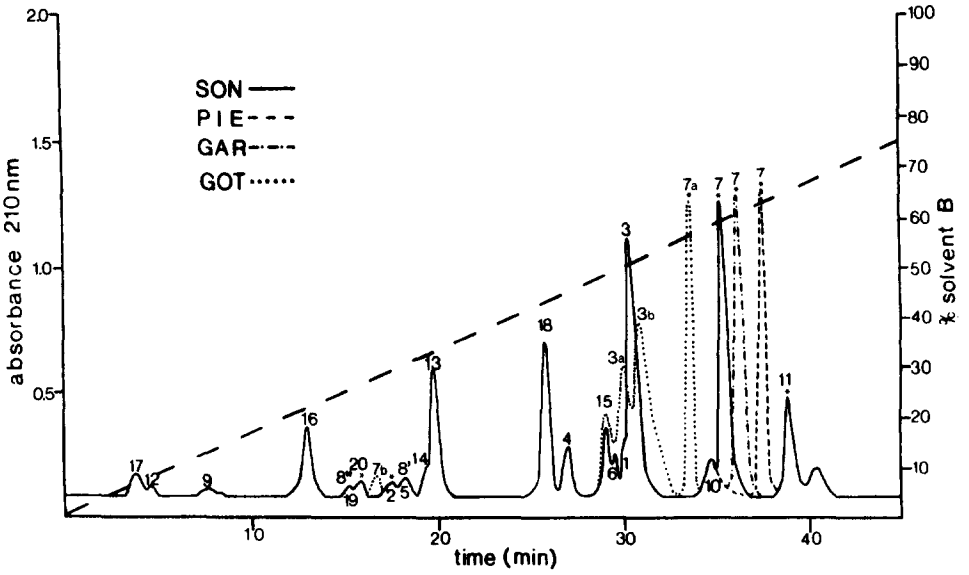

FIGURE 2. Purification of the tryptic peptides of κ chain SON (——), PIE (– – –), GAR (–·–·–·–), and GOT (·····) by HPLC (see text). The asterisk indicates ^{14}C-labeled peptides.

by virtue of an additional prolyl residue located in sequence between positions 95 and 96. The elution profile of peptide T8 (positions 104–107) from proteins PIE and SON could be distinguished from proteins GAR and GOT (Fig. 2). The amino terminal residue of peptide T8′ was valyl for proteins PIE and SON and of peptide T8″ was leucyl for proteins GAR and GOT. Peptide T9 contained a free arginyl residue (Table I) and was assigned on the basis of sequence homology with other κ light chains (3) to position 108, which is located at the end of the V region. The complete V region sequence of κ chains, SON, PIE, GAR, and GOT is presented in Fig. 1.

The light chain constant region (C) residues (positions 109–214) were contained in peptides T10 to T20 (Table II). The complete sequence of peptide T10 (positions 109–126) was determined for protein SON and up to position 116 for the other three proteins; these data are included in Fig. 1. The sequences were identical to that of the C region of human κ chains (3). The amino acid compositions of peptides T10–T20 were identical except for T18′ and T18″. Because this peptide and peptide T14 contain the residues associated with the Km allotypic markers, Km(1), Km(1,2), and Km(3) (15), these two peptides were partially sequenced. Proteins GAR, GOT, and PIE had alanyl and valyl residues at positions 153 and 191, respectively, which is characteristic of the Km(3) allotype, and protein SON, at these same two positions had the alanyl and leucyl residues found for κ chains of the Km(1,2) allotype.

Discussion

Our determination of the complete V region amino acid sequence of light chains obtained from four human IgMκ autoantibodies has provided confirmational data on the remarkably similar primary structure evidenced through partial

sequence analysis of 13 such light chains (10). Proteins GAR, GOT, and SON (shown previously to be virtually identical in sequence to position 61), differed at only few residues of the 108 positions that constitute the entire V_L region. An even closer similarity in sequence among these three κ chains was evident from positions 1–95, which include the portion of the V region encoded by the V gene segment (16, 17). The two RF light chains, GAR and GOT, were identical in sequence except at position 29 and otherwise differed by only one residue from the anti-LDL light chain SON at position 60 located in the third framework region (FR3). Protein SON also contained an additional residue located between positions 95 and 96, the site of combinatorial joining between the V and J (joining) gene segments (16, 17). It is noteworthy that the V segment–encoded sequence of the fourth κ chain, PIE, was completely identical to that of protein GAR even though the two IgM proteins had different antigen binding specificities. The V segment sequence of two other κ light chains from IgMκ RFs, WOL and SIE, determined by Andrews and Capra (8), is also very similar to that of proteins GAR and GOT. The extensive V segment sequence homologies of these κIII chains and of κIII Bence Jones proteins (3) suggest that proteins of this subgroup are encoded by a relatively small number of $V_{\kappa III}$ germ line genes. Indeed, cloning experiments with V_κ gene probes indicate that the number of V_κ germ line genes in the human genome is relatively small, estimated to be <50 (17–19). The nucleotide sequence of the $V_{\kappa III}$ cDNA probe NG9 (18) specifies a V segment amino acid sequence identical to that of protein GAR except for a threonyl/seryl interchange at position 52 in the CDR2, an alanyl/seryl interchange at position 66 in the FR3, and an asparaginyl/seryl and a glutamyl/prolyl interchange at positions 93 and 95, respectively, in the CDR3.

The κIIIb sub-subgroup expresses an antigenic determinant that permits them to be readily distinguished serologically from other chemically defined κIII chains designated κIIIa, or from κIII chains with more extensive V segment sequence differences, e.g., the κIII chain from the IgM RF protein POM (7),[2] which differed from protein GAR by 20 residues. Light chains classified immunochemically as κIII and κIIIb have been identified among Ig molecules from normal individuals, indicating the isotypic nature of the V_κ region subgroup and sub-subgroup (5, 6). The κIII subgroup constitutes almost one-third (30%) of the normal κ chain population of which approximately one-half (45–55%) are κIIIb proteins. Among IgMκ proteins that lacked demonstrable anti-IgG or other types of autoantibody activity, the frequence of occurrence of κIII and κIIIb light chains was found to be similar. In contrast, among 33 anti-IgG, 3 anti-LDL, 1 anti-intermediate filament, and 1 anti-smooth muscle (unpublished data) IgMκ autoantibodies, 36 (95%) had κIII light chains and of this subgroup, 26 (72%) were κIIIb (2, 10). Whether κIII (κIIIb) light chains have a special functional significance to account for their predominance among IgMκ autoantibodies is presently unknown. The CDR1 of κIII (and κI) proteins is characterized by a lesser number of residues than is found for the CDR1 of κII and κIV proteins (3), implying a structural relationship between the configuration of the antigen binding site and the light chain isotype.

[2] The IgMκ RF POM contained the cross-reactive idiotype Po, in contrast to the IgMκ RFs WOL and SIE proteins that shared the cross-reactive idiotype Wa (2).

The major site of sequence difference among the four κ light chains SON, PIE, and GAR, and GOT is at position 96, where each protein possesses a distinctive amino acid residue (Fig. 1). This residue is a transcription product of the nucleotide triplet contributed by V and J genes (16); however, as shown schematically in Fig. 3, alternations in recombination of the 3′ and 5′ ends of the V and J gene segments, respectively, generates additional V_L region diversity and can account for the observed sequence differences, including the extra residue at position 95A of protein SON. The tertiary structural features of the antigen binding site can be profoundly influenced by the chemical nature of the residue at position 96 of the light chain as evidenced from x-ray crystallographic analyses of $F(ab')_2$ fragments and V_L dimers (20, 21). This residue, located in the hapten-binding site of CDR3, also interacts with the comparable residue in the V_H, thereby also affecting heavy-light chain interactions, including their preferential homologous reassociation.

The sequence from positions 97–108 of the J gene segment–encoded region of proteins GAR and GOT was identical and specified by the $J_κ2$ germ line gene; the comparable segments in proteins SON and PIE were products of the $J_κ4$ and $J_κ1$ genes, respectively (22). In addition to the residue at position 96, the J gene–

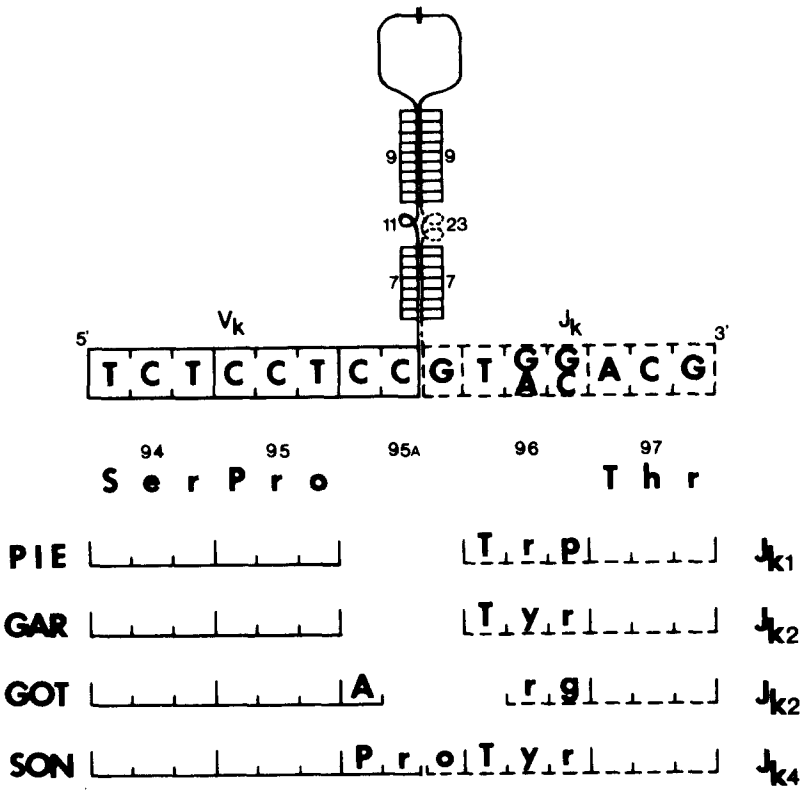

FIGURE 3. Schematic representation of V-J combinatorial joining through pairing of the palindromic sequences of the 3′ non-coding DNA region of the germ line Vκ gene with the 5′ non-coding DNA region of the Jκ genes 1, 2, and 4 (16, 17, 22). The amino acid sequences transcribed by V and J genes are indicated by solid and interrupted lines, respectively.

encoded segment also contains, in FR4, at least two contacting residues involved in V_L-V_H association (20).

The role of the heavy chain in determining the antigen binding specificity of IgM autoantibodies remains to be established. The determination of the complete amino acid V_H sequence of these four and of other monoclonal autoantibodies as well as the production of antiidiotypic antibodies (in progress in our lab) should provide further insight into the structural features responsible for the preferential association of light and heavy chain isotypes, the expression of idiotypic determinants, and the chemical nature of the antigen binding site. The preferential association of particular light and heavy chains among human monoclonal IgM autoantibodies implies a genetic control of the immune response to self antigens. Jerne (23) has proposed that specific isotypic and/or idiotypic features of homogeneous Igs participate in a network of cellular interactions by which the immune response is regulated. Support for this hypothesis has come from the demonstration of cross-reactive idiotypes in the autoimmune response elicited in inbred mice (24–27) and among human autoantibodies (e.g., the Wa and Po cross-reactive idiotypes present among monoclonal IgM RFs) (2). Continued studies of monoclonal IgM autoantibodies should provide further insight into the structural, genetic, and evolutionary factors that control the immune response to self antigens.

Summary

Light chains of the serologically and chemically defined V region sub-subgroup κIIIb are preferentially associated with several types of human IgMκ (monoclonal) autoantibodies and are remarkably homologous in primary structure, as evidenced by partial amino acid sequence data. To establish the extent of homology among such proteins, we have determined the complete variable region (V) sequence of the light chains of four monoclonal IgMκ autoantibodies, of which two (GAR and GOT) are rheumatoid factors (RFs), the third (SON) has anti-apo β lipoprotein specificity, and the fourth (PIE) binds specifically to intermediate filaments. The region encoded by the V_κ segment gene (positions 1–95) in all four light (L) chains is virtually identical in sequence, differing by only one residue in the FR3 of protein SON and in the first CDR of protein GOT. Further, the CDR3 of κ chain SON contains an additional residue (prolyl) located at the carboxyl-terminus of the V segment. The region encoded by the J gene (positions 96–108) is identical after position 96 for the two RFs GAR and GOT ($J_\kappa 2$), but different in proteins SON ($J_\kappa 4$) and PIE ($J_\kappa 1$). The amino acid residue at position 96, located in CDR3 at the site of combinatorial joining of the V_κ and J_κ gene segments and involved as a contacting residue in the hapten binding site, is different in all four light chains. These results demonstrate the extensive homology in sequence among light chains of IgMκ autoantibodies and indicate that a particular V_κ germ line gene, κIIIb, is expressed as a phylogenetic response to certain self antigens or as part of a selection process by which these autoimmune responses are regulated.

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