

MOLECULAR GENETIC ANALYSIS OF THE V_κSer GROUP ASSOCIATED WITH TWO MOUSE LIGHT CHAIN GENETIC MARKERS

Complementary DNA Cloning and Southern Hybridization Analysis

By MARIANNA M. GOLDRICK, R. THOMAS BOYD, PAUL D. PONATH,
SHUN-YING LOU, AND PAUL D. GOTTLIEB

From the Department of Microbiology, University of Texas, Austin, Texas 78712

The extensive amino acid sequence diversity of Ig variable (V)¹ regions is known to be derived from both evolutionary and somatic processes (1–3). Comparison of amino acid sequences of mouse myeloma and hybridoma κ light (L) chains, for example, indicates that these sequences can be arranged into groups such that members of a group are more similar to each other than to members of other groups (4–6). In the case of mouse κ L chains, multiple V region genes have been observed (7, 8) for most groups of V regions tested. Thus, the multiple germ line V_κ genes provide a portion of the V region diversity observed at the protein level. Additional diversity is introduced by permitting each V_κ gene to join with any one of four different active joining (J) segments, by introducing junctional diversity during the joining event, and by somatic mutation (for review, see refs. 3 and 9).

Several mouse V_κ genetic markers have been described which involve characteristic V_κ regions that are expressed by some inbred strains of mice, but not by others. These genetic markers have been detected by peptide mapping (10, 11) and isoelectric focusing (12, 13) of L chains from normal mouse serum Ig, by analysis of expression of various idiotypes (14, 15), and by isoelectric focusing of L chains of antiphosphorylcholine antibodies that bear the H8 idiotypic (16). All of these genetic polymorphisms are determined by genes closely linked to the *Lyt-2* and *Lyt-3* loci on chromosome 6 (17, 18), the same chromosome that bears the κ L chain structural genes (19). A system for nomenclature of genes that determine these V_κ polymorphisms has been proposed (20).

One of these V_κ polymorphisms, called the I_B-peptide marker, was detected by tryptic peptide mapping of reduced and [¹⁴C]iodoacetic acid-alkylated L chains from normal serum Ig of unimmunized mice (10, 11). This analysis detected a heterogeneous group of V_κ regions that accounts for <5% of the total L chains expressed by I_B-positive strains. To obtain myelomas producing homogeneous

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¹ *Abbreviations used in this paper:* C, constant region of Ig; cDNA, complementary DNA; CDR, complementarity-determining region; Fr, framework; J, joining region of Ig; L, light chain of Ig; NP, (4-hydroxyl-3-nitrophenyl)acetyl; 5'UT, 5' untranslated region; V, variable region of Ig.

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examples of I_B-positive L chains, it was necessary to cross the genetic marker onto the BALB/c genetic background, since none of the myeloma-inducible strains expresses the marker (21). Of ~200 myelomas induced in the C.C58 and C.AKR strains, several were found to correspond to both the I_B-peptide marker and the EF1^a marker described by Gibson et al. (12). Amino acid sequence analysis demonstrated that these L chains belonged to a group of V_κ regions apparently not expressed by either BALB/c or NZB, and which we referred to as the V_κSer group, because all such proteins observed had an unusual amino-terminal serine residue (21). A major question that remains to be answered is whether strains that do not express V_κSer-like L chains lack V_κSer genes altogether, or whether they contain the genes, but fail to express them due to regulatory controls.

To further characterize the V_κSer group, full-length complementary DNA (cDNA) clones have been produced from mRNA coding for the I_B-positive L chain of the C.C58 M75 myeloma. The entire sequence of one cDNA clone has been determined and compared with the known collection of BALB/c and NZB κ chain sequences. Results suggest that the nucleotide sequence of the M75 V region is different from all known V_κ regions throughout most of its length. Use of a the 5' region of this clone as a probe for V_κSer-specific sequences in Bam HI, Hind III, and Eco RI digests of liver DNA of a large number of inbred, recombinant, and recombinant inbred strains has revealed at least one strongly hybridizing sequence in each strain tested. Thus, strains that do not express V_κSer-associated phenotypic markers do not lack V_κSer-related DNA sequences. However, strains could be divided into two groups based on the sizes of the strongly-hybridizing fragments. Complete correlation was observed between expression of V_κSer-associated phenotypic markers and the presence of strongly hybridizing Bam HI, Hind III, and Eco RI fragments of 3.2 kilobases (kb), 2.8 kb, and 2.1 kb, respectively.

Materials and Methods

Mice, Myelomas, and DNA. The C.C58 and C.AKR inbred strains of mice, which bear the *Lyt-2^a* and *Lyt-3^a* alleles and V_κ genetic polymorphisms of the C58/J and AKR/J strains, respectively, on the BALB/cAn genetic background (22), and the B6/*Lyt-2^a*, *Lyt-3^a* strain, which contains the corresponding genes of the RF strain on the C57BL/6 background (23), were bred in our laboratory. The C.C58 and C.AKR strains were inbred after 19 backcross generations. Mice of the congenic partner strain, BALB/cAn, were the gift of Dr. H. N. Eisen, Massachusetts Institute of Technology, Cambridge. Mice of the B6.PL(85NS) strain (24) and DNA from mice of the NAK strain (25) were the gifts of Dr. D. Gibson, University of Sherbrooke, Quebec, Canada. All other mice were purchased from the Jackson Laboratory, Bar Harbor, ME.

The C.C58 M75 myeloma was induced in an earlier inbreeding (N9) by three bimonthly intraperitoneal injections of pristane (tetramethylpentadecane), as described by Potter (26). The tumor had been adapted to subcutaneous growth, and small nonnecrotic tumors, ~1.5 cm in diameter, were quick-frozen in liquid nitrogen for mRNA isolation. DNA from the C.C58 M75 myeloma and from livers of various strains was isolated from tissue frozen in the same manner. DNA from A/J mice and from AKXL recombinant inbred strains (27) was purchased from The Jackson Laboratory, Bar Harbor, ME.

Bacterial Strains and Vectors. The primer and linker vectors (28) in *E. coli* K12 HB101 were the kind gifts of Dr. H. Okayama and Dr. P. Berg, Stanford University School of Medicine. The plasmid pUC18 (29) and *E. coli* K12 GM33 (30) were obtained from Dr.

R. Meyer, University of Texas, Austin. The M13mp8 and M13mp9 phage, and *E. coli* K12 JM103 strain (31) were obtained from Dr. J. Walker, University of Texas, Austin. The RR1 strain of *E. coli* K12 and the pBR322 clone, which contained the 3.1 kb Hind III/Bam HI BALB/c genomic C_{κ} (constant) fragment (32, 33) were the gifts of Dr. P. Tucker, University of Texas Health Sciences Center, Dallas.

Enzymes. Restriction enzymes were purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, MD), Boehringer-Mannheim Biochemicals (Indianapolis, IN), and New England Biolabs (Beverly, MA). Avian myoblastosis virus reverse transcriptase was purchased from Life Sciences, Inc., St. Petersburg, FL. *E. coli* DNA polymerase I (Klenow fragment), T4 DNA polymerase, and a kit for radiolabeling DNA by strand-replacement synthesis with T4 DNA polymerase were purchased from Bethesda Research Laboratories, Inc. *E. coli* DNA ligase was purchased from P-L Biochemicals, Piscataway, NJ. A kit (409 Sequencing Pack) for nucleotide sequence analysis using the dideoxynucleotide procedure was obtained from New England Biolabs, Beverly, MA.

Isolation of mRNA. PolyA⁺ RNA was isolated from the C.C58 M75 myeloma by the guanidine thiocyanate method of Chirgwin et al. (34), followed by two passages over oligo(dT)-cellulose (Sigma Chemical Co., St. Louis, MO). Electrophoresis in formaldehyde gels, and Northern blot analyses were performed as described by Rave et al. (35), and hybridization in 50% (vol/vol) formamide was as described by Thomas (36).

Probe for Screening cDNA Library. A pBR322 subclone was obtained from Dr. P. Tucker. It contained a 3.1 kb Hind III/Bam HI fragment containing the BALB/c mouse C_{κ} region, and a portion of 5' and 3' flanking regions (32, 33). The 3.1 kb fragment was excised and isolated by two cycles of agarose gel electrophoresis, and radiolabeled with α -[³²P]dATP (New England Nuclear, Boston, MA, 3,200 Ci/mmol) by nick-translation, as described by Maniatis et al. (37). Specific radioactivity of the resulting DNA was $\sim 5 \times 10^6$ cpm/ μ g.

Procedure for cDNA Cloning. Complementary DNA cloning of polyA⁺ RNA from the C.C58 M75 myeloma was performed essentially as described by Okayama and Berg (28). After second-strand synthesis and ligation, the resulting DNA was used to transform both *E. coli* K12 strain HB101 (Rec A⁻) and RRI (Rec A⁺) as described and plated on L-broth agar that contained 250 μ g/ml Carbenicillin (Geopen) (Pfizer, Inc., New York). Colonies were picked onto nitrocellulose filters (Millipore Corp., Bedford, MA) and subjected to colony hybridization as described by Davis et al. (38), using the nick-translated Hind III/Bam HI genomic C_{κ} fragment as probe. Colonies that gave positive hybridization signals were grown in L broth containing 250 μ g/ml carbenicillin, and plasmid DNA was isolated by a cleared lysate procedure, essentially as described by Katz et al. (39).

Restriction Mapping. For restriction mapping of cloned DNA, restriction enzymes were generally used under the conditions specified by their suppliers. Fragments were analyzed by electrophoresis on agarose gels (0.8–1.5%) in the presence of ethidium bromide.

Southern Hybridization of Genomic DNA. Genomic DNA was isolated from myeloma and liver tissue as described by Blattner et al. (40) and Maniatis et al. (37). DNA samples (10 μ g each) were digested with the appropriate restriction nuclease and subjected to electrophoresis on 0.6% agarose gels. The DNA was transferred to nitrocellulose filters (BA85; Schleicher and Schuell, Keene, NH), prehybridized, then hybridized with radioactive probe (see below) in 50% formamide at 41°C, essentially as described by Davis et al. (38). Filters were washed three times (10 min each) at 55°C in 2 × SSC (standard sodium citrate), 0.1% SDS followed by two washes (15 min each) at 55°C in 0.1 × SSC, 0.1% SDS. Autoradiography was performed at -70°C with Dupont Lightning Plus intensifying screens.

The source of probe for Southern hybridization was a 200 base pairs (bp) Pst I/Kpn I DNA fragment that contained the 5' end of the putative full-length κ L chain cDNA clone of interest, p9(35) (see below). This fragment was subcloned into the plasmid, pUC18(29), and the resulting plasmid was cleaved at one end of the insert with Pst I and radiolabeled with α -[³²P]dATP (sp act 3,000 Ci/mmol) by strand-replacement synthesis with T4 DNA polymerase as described by O'Farrell (41). Specific radioactivity of the resulting probe was approximately $\sim 10^8$ cpm/ μ g DNA.

DNA Sequence Analysis. DNA sequencing was performed by the dideoxynucleotide chain termination method of Sanger and coworkers (42) using cloning vectors M13mp8 and M13mp9 and the sequencing strategy designed by Messing and coworkers (31, 43, 44). Replicative form of M13 clones was prepared by the rapid boiling procedure described by Maniatis et al. (37) for characterization of inserts. Single-stranded form of M13 clones was prepared by a modification of the procedure of Schreier and Cortese (45) as described by New England Biolabs (sequencing kit 409; Beverly, MA). Sequencing reactions uses *E. coli* DNA polymerase I, Klenow fragment, M13 primer (pentadecamer) fragment, and mixtures of each of the four dideoxynucleotides with normal nucleotide precursors (New England Biolabs), as well as α -[32 P]dATP (800 Ci/mmol; New England Nuclear) as a source of label. Gel electrophoresis was on thin polyacrylamide gels, essentially as described by Sanger and Coulson (46).

Results

Production of cDNA Clones. Total poly(A)⁺ RNA was isolated from the C.C58 M75 myeloma and subjected to formaldehyde gel electrophoresis. Hybridization with the 32 P-labeled, nick-translated 3.1 kb C_{κ} probe (see Materials and Methods) demonstrated a single hybridizing 1 kb species, approximately the size expected for a kappa L chain mRNA. Complementary DNA clones of the total poly(A)⁺ mRNA were made by the method of Okayama and Berg (28). Transformants were screened by colony hybridization with the 3.1-kb C_{κ} probe, and 11 strongly hybridizing colonies were identified, all in the RR1 strain. Three of these were shown by restriction enzyme mapping to contain inserts of \sim 1,050 bp, large enough to encode the entire M75 L chain and 360 bp of flanking sequences.

The restriction map of the mouse DNA insert in plasmid p9(35) and the strategy employed in determining its nucleotide sequence are illustrated in Fig. 1. The presence of a Bcl I site was suspected from work of Max et al. (47), and to use it for cloning into M13mp6 and M13mp9, plasmid p9(35) was isolated after transformation of *E. coli* K12 GM33 that lacks the *dam* methylase system (30). To analyze the sequence at the 3' end of the insert, a fragment of p9(35) was cleaved with Pvu II and treated with the enzyme Bal-31 for various periods, as described by Poncz et al. (48). Samples at each time point were cleaved with Bcl I, and the fragments corresponding to the 3' end of the cDNA were cloned

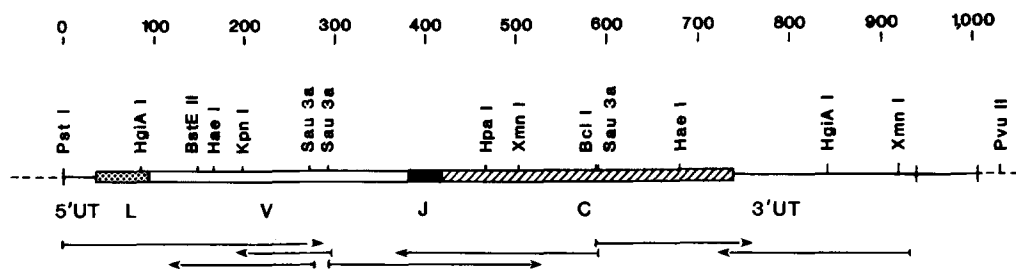


FIGURE 1. The strategy employed in determining the nucleotide sequence of the cDNA insert of plasmid p9(35) is indicated by the arrows beneath the figure. Sequence from right to left (3' to 5' of the mRNA) was determined from M13mp8 clones, and from left to right (5' to 3' of the mRNA) was determined from M13mp9 clones. Coding segments are indicated as follows: L, ▨; V, □; J, ■; C, ▩. The 5' and 3' untranslated regions appear as a solid line (—), and vector is indicated by a dashed line (-----). The nucleotide sequence of the 3' untranslated region and poly(dAdT) segment from position 938 to approximately position 1,004 was not determined (see text).

into the *Bcl* I and *Hinc* II sites of M13mp8 for sequence analysis. The nucleotide sequence of 938 bp of the L chain cDNA insert of p9(35) is presented in Fig. 2. The sequence of ~66 bp at the extreme 3' end of the 3' untranslated region and the poly(dAdT) region, estimated at 50–60 bp, was not determined.

Southern Hybridization of Genomic DNA Fragments. High molecular weight DNA from the C.C58 M75 myeloma and from livers of the C.C58, C58/J, and BALB/cAn strains of mice was digested with *Bam* HI, *Hind* III, or *Eco* RI and subjected to electrophoresis on 0.6% agarose gels. The DNA was transferred to nitrocellulose and hybridized to a ³²P-labeled probe consisting of a 200 bp *Pst* I-*Kpn* I fragment that contained the 5' untranslated region (5'UT), leader, first framework (Fr1), and first complementarity determining region (CDR1) of the M75 κ L chain. This fragment had been cloned into the plasmid pUC18 (29) and radiolabeled as described in Materials and Methods.

Comparison of the *Bam* HI and *Hind* III fragments labeled in C.C58 M75 myeloma DNA with those in C.C58 liver DNA reveals in both digests a unique hybridizing fragment in the M75 DNA (Fig. 3*a*). Further studies not reported here (M. Goldrick, P. Ponath, and P. Gottlieb, unpublished results) indicated that these fragments (7.4 and 4.0 kb in the *Bam* HI and *Hind* III digests, respectively) corresponded to the rearranged L chain gene expressed by this myeloma. The fragment shared by the M75 myeloma and C.C58 liver DNA (3.2 and 2.8 kb in *Bam* HI and *Hind* III digests, respectively) is likely to correspond to an unrearranged germ line gene homologous or identical to that expressed by the M75 myeloma. As expected, liver DNA from the C58/J strain, the source of the L chain genes of the C.C58 strain (22), contains similar common fragments. Of major interest is the finding that the *Bam* HI and *Hind* III digests of liver DNA from BALB/cAn, the strain that provided the genetic background of C.C58, and which does not express the genetic markers shown to be due to M75-like L chains (21, 22), contain strongly hybridizing fragments of 7.8 and 8.4 kb, respectively. V_{κ} Ser-related fragments of different size were also observed in *Eco* RI digests of C.C58 and BALB/c DNA (2.1 and 2.8 kb, respectively) (Fig. 3*b*).

Liver DNA from a variety of inbred, recombinant-inbred, and recombinant strains was then digested with *Bam* HI, *Hind* III, or *Eco* RI, and tested in Southern hybridizations with the V_{κ} Ser-specific probe. As summarized in Table I, all strains that express the I_{B} -peptide marker and the $Ef1^a$ polymorphism contain strongly-hybridizing fragments of the size present in the C.C58 and C58/J strains (Fig. 3, *a* and *b*). Strains that do not express these markers contain hybridizing fragments identical in size to those of the nonexpressing strain, BALB/cAn.

Discussion

The nucleotide sequence of the 5'UT, leader, and V segments of the C.C58 M75 κ chain cDNA clone p9(35) is aligned in Fig. 2 with the sequences determined for the BALB/c myeloma kappa chains MOPC21 (49) and MPC11 (50). These are two of the BALB/c myeloma κ chains to which the M75 L chain is most homologous (6). To maximize homology, the 36-bp insertion in the V segment of the MPC11 sequence (50) has been omitted from the sequence (see Fig. 2 legend). The percentage differences observed when nucleotide sequences

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      5'UT
      10
      (-29)
      50
      -20
M75   gcgcgggg   ata cat cag gca ggc aaa ggc atc aag atg aag tca cag acc cag gtc ttc ata ttt
MOPC21  NNN   --g --- -- a-c a-- -tg --- --- --- g-a --- --- -t -t- --- --- -cc
MPC11   --g --- --c a-c a-- -tg --- --- --- -a --- --- -tt --- --- -t g-- --c

      +1
      100
      +10
M75   cta ctg ctc tgt gtg tct ggt gct cat ggg agt att gtg atg acc cag act ccc aaa ttc ctg
MOPC21  a-- --- --- --g t-a -a- --a --- g-- --- -ac --- --a --- --- --a t-- --- --- -c- a--
MPC11   g-g t-t --- --g t-- --- --- -t- g-c --a gac --- --- --- --- t-- -a- --- --- a--

      150
      +20
      CDR1
      +30
M75   cct gta tca gca gga gac agg gtt acc atg acc tgc aag gcc agt cag agt gtg ggt aat aat
MOPC21  t-c a-g --- -t- --- --g --- --c --- t-- --- --- --- --- --- g-- -a- --- -t- -c- t--
MPC11   t-c ac- --- -t- --- --- --- --c -g- --c --- --- --- --- --- ga- --- a-- -c- -c-

      200
      +40
      CDR2
      +50
M75   gta gcc tgg tac caa cag aag cca gga cag tct cct aaa ttg ctg ata tac tat gca tcc aat
MOPC21  --t t-N --- --t --- --a --- --a --- -ag --- --- --- --- --- c-- --c --- --- --t ggg --- --- -c-
MPC11   --g --- --- --t --g --- --a --- --g --a --- --- --- --- c-a --- --t --t tc- --- --- t-c

      300
      +60
      +70
M75   cgc tac act gga gtc cct gat cgc ttc act ggc agt gga tct ggg aca gat ttc act ttc acc
MOPC21  --g --- --- --g --- --N --- --- --- --a --- --- --- --- -ca --- --- --- --- c-g ---
MPC11   --g --- --- --- --c --- --- --- --N --- --- --- --- --- --g --- --- --- --- --g ---

      350
      +80
      CDR3
      +90
M75   atc agc agt gtg cag gtt gaa gac ctg gca gtt tat ttc tgt cag caa cat tat acc tct cct
MOPC21  --- --- --- --- -c- --- --- --t --- -a- --- ca- --- gga --g gg- --c -g- -a- --g
MPC11   --- --- --- --- -c- --- --- --a --- --- --a --- --- --- --- -a- --- --- --- -gt a- ---

      400
      +100
      +110
M75   oeg acg ttc ggt gga ggc acc aag ctg gaa atc aat cgg gct gat gct gca cca act gta tcc
      tg
      a

      450
      +120
      +130
M75   atc ttc cca cca tcc agt gag cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac

      500
      +140
      +150
M75   aac ttc tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga caa aat ggc

      550
      +160
      +170
M75   gtc ctg aac agt tgg act gat cag gac agc aaa gac agc acc tac agc atg agc agc acc ctc

      600
      +180
      +190
      +200
M75   acg ttg acc aag gac gag tat gaa cga cat aac agc tat acc tgt gag gcc act cac aag aca

      650
      +210
      3'UT
      750
M75   tca act tca ccc atc gtc aag agc ttc aac agg aat gag tgt tagagacaaaggtcctgagatgccacc
      t
      c

      800
M75   accagctccccagctccatcctatcttcccttctaaggcttggaggcttccccacaagcgacctaccactgttgcggtgctc

      850
      900
M75   caaacctcctccccacctccttctcctcctcctcccttcttggcttttatcatgctaataatttcagaaaaattcaataa

M75   agtgagtc

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of Fr and CDR of the M75, MOPC21, and MPC11 L chains are compared pairwise are shown in Table II. The Fr1, Fr2, CDR1, CDR2, and leader plus 5'UT regions of M75 differ from the corresponding regions of MOPC21 and MPC11 by comparable amounts, ranging from 16 to 28%. In contrast, Fr3 of M75 is more closely related, differing at 13 and 5% of its nucleotides from the corresponding region of MOPC21 and MPC11, respectively. Comparison of the MOPC21 and MPC11 sequences indicate comparable differences in most segments.

The translated amino acid sequence of the C.C58 M75 L chain is compared in Fig. 4 with the amino acid sequences of BALB/c L chains, which appear to be most closely related to M75 (6). It is immediately apparent that both MOPC21 and MPC11 contain leader sequences that are nine amino acids longer than that of the M75 L chain. As summarized in Table III, large differences (39–40%) are observed between the leader and Fr1 segments of the M75 L chain and those of MOPC21 and MPC11 at the positions over which they can be compared. In contrast, the Fr2 region of M75 is nearly identical to those of MOPC21 and MPC11, differing at only 7 and 0% of the amino acids, respectively. The amino acid sequence of Fr2 of M75 is more similar to the corresponding region of MOPC21 and MPC11 than would be expected from differences at the nucleotide level (16% and 20%, respectively). This is also evident when the Fr2 regions of MOPC21 and MPC11 are compared. One interpretation of this finding is that selection at the protein level, perhaps for ability of the L chain to fold properly or to associate with H chain, may be acting on the Fr2 region. In contrast to Fr2, differences in amino acid sequence between M75 and the other proteins in Fr3 are what might be expected from their nucleotide sequences.

The partial amino acid sequence most like that of M75 is that of the TEPC1017 κ chain (5, 52, 53) (Fig. 4). Only five differences between M75 and TEPC1017 are observed in the Fr1 region; three fewer than the next most closely related protein. Southern hybridization analysis of TEPC1017 myeloma DNA is in progress to determine whether the expressed V_{κ} region has significant homology with the M75 V_{κ} sequence.

The J segment of the C.C58 M75 κ chain is nearly identical in nucleotide sequence to that of $J_{\kappa 1}$, the J segment farthest upstream of the BALB/c C_{κ} gene (33, 54). The differences from BALB/c $J_{\kappa 1}$ at positions 381 and 382 (Fig. 2) may reflect junctional diversity introduced at position 96 of the L chain by joining V

FIGURE 2. The sequence of nucleotides 1–938 of the cDNA insert of plasmid p9(35), which codes for the entire C.C58 M75 κ chain, is shown. Arrows mark the beginning of the 5'UT, leader (L), V, J, C, and 3' untranslated (3'UT) regions. CDR1, 2, and 3 are as designated according to Kabat et al. (6). Shown for comparison are the nucleotide sequences of the 5'UT, leader, and V segments of the BALB/c MOPC21 (49) and MPC11 (50) myeloma κ chains. The 36 bp insertion present after nucleotide 114 in the MPC11 L chain sequence (50) has been omitted to allow comparison with the other L chains. Positions in MOPC21 and MPC11 that are identical to M75 are indicated by a dash (—). Positions where the sequence is not known are indicated by N. The ATG codon, which begins the L segment of M75, is numbered –20, and the last codon of the C region is numbered +214. The L segments of the MOPC21 and MPC11 L chains are nine amino acids longer than that of M75, and begin at the codon numbered –29. Shown in boldface type are the nucleotides in M75 that differ from the germline sequences of BALB/c as determined by Max et al. (47, 54). The nucleotides present at the corresponding positions in BALB/c are shown beneath the M75 sequence.

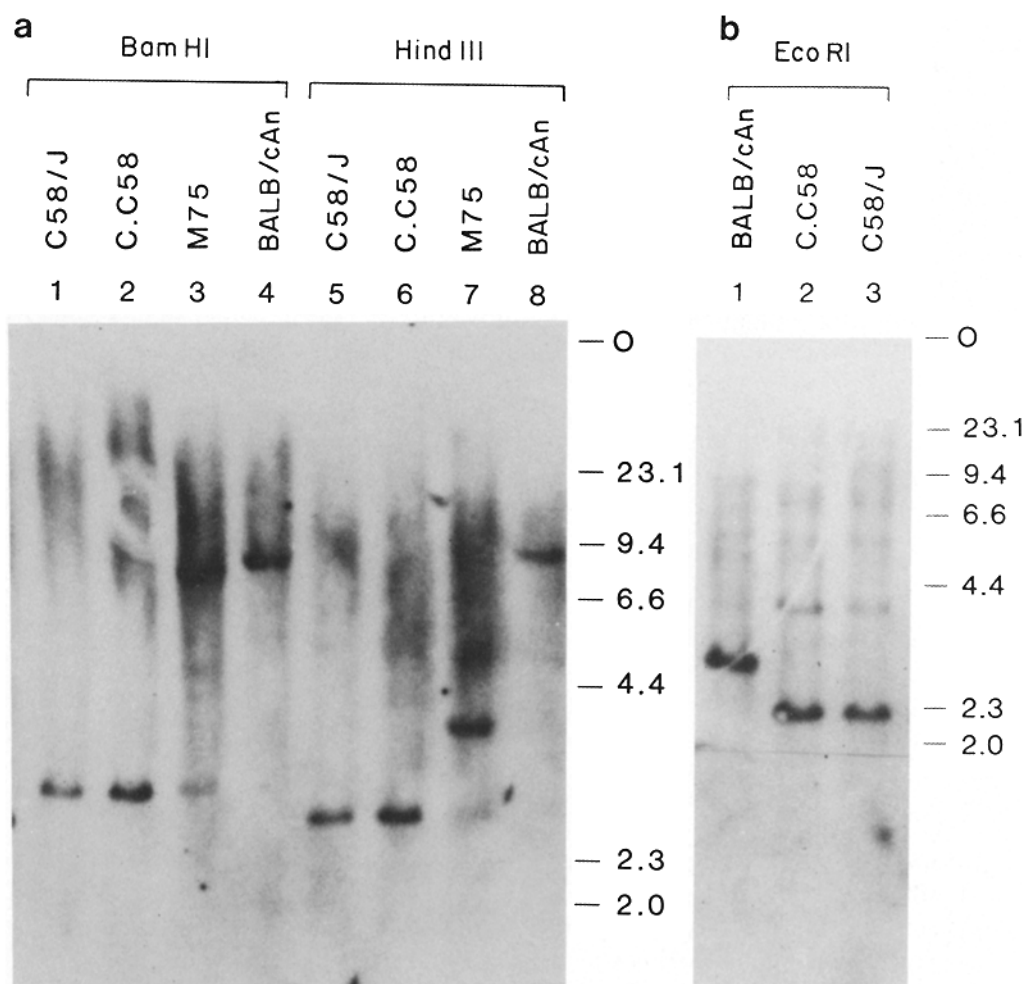


FIGURE 3. Southern hybridization analysis of genomic DNA from the C.C58 M75 myeloma and from liver of the C.C58, C58/J, and BALB/cAn strains was performed as described in Materials and Methods. (a) Digestion was performed with Bam HI (1-4) and Hind III (5-8) on the following DNA samples: C58/J liver DNA (1 and 5); C.C58 liver DNA (2 and 6); C.C58 M75 myeloma DNA (3 and 7); and BALB/cAn liver DNA (4 and 8). The sizes of molecular weight standards are given in kb; (b) Digestion was performed with Eco RI on BALB/cAn, C.C58, and C58/J liver DNA (1, 2, and 3, respectively).

and J gene segments. The difference at nucleotide 416, which results in substitution of an asparagine at position 107 in M75 for a lysine in BALB/c could reflect a difference in the germ line J sequences of BALB/c and C.C58 strains, or somatic variation in the expressed M75 L chain gene. The basis of these differences will become clear from nucleotide sequence analysis of the C.C58 germ line J_κ genes.

Comparison of the nucleotide sequence of the C_κ region of the C.C58 M75 L chain with that of the unrearranged BALB/c C_κ gene (47) revealed a single silent substitution in the codon for isoleucine 205 of the M75 L chain (Fig. 2). The 3' untranslated region of the M75 L chain cDNA differs in sequence from the

TABLE I
Correlation of Expression of I_b -peptide and $Ef1^a$ Phenotypes with Size of Restriction Fragments
Containing V_{κ} Ser-related Genes

Strain	I_a	$Ef1^a$	Bam HI		Hind III		Eco RI	
			3.2*	7.8	2.8	8.4	2.1	2.8
C.C58	+	+	+	-	+	-	+	-
C58/J	+	+	+	-	+	-	+	-
PL/J	+	+	+	-	+	-	+	-
RF/J	+	+	+	-	+	-	+	-
B6/Lyt-2 ^a , Lyt-3 ^a	+	+	+	-	+	-	+	-
AKR/J	+	+	+	-	+	-	+	-
C.AKR	+	+	+	-	+	-	+	-
NAK	NT [‡]	+	+	-	+	-	NT	NT
AKXL 13	NT	+	+	-	NT	NT	+	-
AKXL 24	NT	+	+	-	NT	NT	+	-
AKXL 25	NT	+	+	-	NT	NT	+	-
AKXL 28	NT	+	+	-	NT	NT	+	-
BALB/cAn	-	NT	-	+	-	+	-	+
BALB/cJ	-	-	-	+	-	+	-	+
C57Bl/6J	-	-	-	+	-	+	-	+
SJL/J	-	-	-	+	-	+	-	+
CBA/J	-	-	-	+	-	+	-	+
SWR/J	-	-	-	+	-	+	-	+
C3H/HeJ	-	NT	-	+	-	+	-	+
A/J	-	-	-	+	-	+	-	+
B6.PL(85NS)	NT	-	-	+	NT	NT	-	+
AKXL 5	NT	-	-	+	NT	NT	-	+
AKXL 6	NT	-	-	+	NT	NT	-	+
AKXL 7	NT	-	-	+	NT	NT	-	+
AKXL 8	NT	-	-	+	NT	NT	-	+
AKXL 9	NT	-	-	+	NT	NT	-	+
AKXL 12	NT	-	-	+	NT	NT	-	+
AKXL 14	NT	-	-	+	NT	NT	-	+
AKXL 16	NT	-	-	+	NT	NT	-	+
AKXL 17	NT	-	-	+	NT	NT	-	+
AKXL 19	NT	-	-	+	NT	NT	-	+
AKXL 21	NT	-	-	+	NT	NT	-	+
AKXL 29	NT	-	-	+	NT	NT	-	+
AKXL 37	NT	-	-	+	NT	NT	-	+
AKXL 38	NT	-	-	+	NT	NT	-	+

Most of the I_b -peptide and $Ef1^a$ phenotypes have been reported previously (10, 18). The $Ef1$ phenotypes of B6.PL(85NS) and NAK mice were determined by Gibson et al. (24, 25).

* Restriction fragment lengths in kb.

[‡] NT, not tested.

[§] The AKXL 29 strain was originally typed as $Ef1^a$ -positive (18). The strain then underwent a breeding crisis, and was outcrossed to the C57L/J strain. Progeny were backcrossed four times to AKXL 29, and inbreeding of the resulting mice resulted in fixation of the AKXL 29 strain for the $Ef1^b$ allele (B. A. Taylor, The Jackson Laboratory, personal communication).

region 3' to the embryonic BALB/c C_{κ} gene only at nucleotide 758 (Fig. 2). If the C.C58 C_{κ} gene is derived from the C58/J strain, then the nearly complete conservation of this region suggests that it may determine some important function.

TABLE III
*Comparison of Amino Acid Sequences of Leader, Fr, and CDR of
 M75, MOPC21, and MPC11 L Chains*

	Amino acid differences between:		
	M75 and MOPC21	M75 and MPC11	MOPC21 and MPC11
Leader	40	45	31
Fr1	39	39*	30*
CDR1	55	36	46
Fr2	7	0	7
CDR2	14	29	29
Fr3	16	6	13
CDR3	56	22	44

Segments compared are defined with respect to Fig. 4 as follows: leader, -20- -1; Fr1, 1-23; CDR1, 24-34; Fr2, 35-49; CDR2, 50-56; Fr3, 57-88; CDR3, 89-97.

* The 12-amino acid segment that precedes residue 1 of the MPC11 L chain and results from a 36-bp insertion (50) has been omitted in these comparisons.

hybridizing Bam HI, Hind III, and Eco RI fragments of 3.2, 2.8, and 2.1 kb, respectively, was found to correlate completely with expression by the strain of the I_B-peptide and Ef1^a markers (Table I). All nonexpressor strains had strongly hybridizing Bam HI, Hind III, and Eco RI fragments of 7.8, 8.4, and 2.8 kb, respectively. In addition, in some Southern hybridizations (e.g. Fig. 3*b*) more weakly hybridizing bands are observed with DNA from strains of both groups, and preliminary results suggest that the size distribution of these bands may also be characteristic of each group. Whether or not these bands contain genes belonging to the same V_κ gene family as the strongly-hybridizing fragment is not yet known. Finally, it is not known whether differences in flanking regions that give rise to the restriction polymorphisms observed also determine expression of V_κSer L chains by one group of strains and not by the other. Analysis of the DNA surrounding the V_κSer-like genes in strains of both groups should shed light on this question, and may provide clues to the genetic events that resulted in divergence of the two classes of strains in this chromosomal region.

Until this study, one possible explanation for expression of L chain phenotypic markers associated with the V_κSer group was that strains that do not express these markers lack V_κSer-like genes altogether. Siekevitz et al. (55) have shown that strains that do not express the major crossreactive idiotype of antiarsonate antibodies of A/J mice lack the single strongly hybridizing V_H gene present in idiotype-positive strains. This is not the case for strains that do not express V_κSer-associated markers, as shown by our Southern hybridization experiments, which show strongly hybridizing sequences in all strains tested. A second possible explanation is that the V_κSer-like genes present in strains that do not express the I_B-peptide and Ef1^a markers contain differences in the coding regions that alter the structure of the products so that they are undetectable in the assays for the phenotypic markers. If this were so, their products might nevertheless be expected to occur at random in the large BALB/c myeloma collection (6). As

shown here, even the most closely related BALB/c myeloma or hybridoma κ chains observed differ markedly from the M75 L chain (Figs. 2 and 4). The frequency of expression of the BALB/cAn V_{κ} Ser-like L chain genes would appear to be very low. In contrast, V_{κ} Ser-like L chains were observed with an incidence of at least 7 out of a total of ~ 200 C.C58 and C.AKR myelomas induced (21). Results of studies in progress (J. Bednarczyk and P. Gottlieb, unpublished results) using the V_{κ} Ser-specific probe to analyze BALB/c hybridomas by the lysate hybridization method of Manser and Geffer (56) should provide information on the expression of V_{κ} Ser-like genes in this strain.

It is possible that V_{κ} Ser-like genes of strains that do not express V_{κ} Ser-associated markers contain in-frame termination codons in coding regions or mutations in promoter, splicing, recombination, or other signals required for their expression. Nucleotide sequence analysis of cloned unrearranged V_{κ} Ser-like genes from liver DNA of both BALB/cAn and C.C58 strains should show whether or not V_{κ} Ser-like genes of BALB/cAn are defective in coding or regulatory sequences, or conversely, whether C.C58 V_{κ} Ser-like genes contain unusual flanking sequences that may affect their expression. These studies are in progress. Only one strongly hybridizing fragment and one V_{κ} Ser-like gene has been identified to date in each strain (M. Goldrick, T. Boyd, P. Ponath, and P. Gottlieb, unpublished results). However, multiple clones must be analyzed, as shown by results of Siekevitz et al. (57), who isolated three different V_H genes related to the V_H gene that determines the major crossreacting anti-Ars idiomotype of A/J mice from a single hybridizing 6.4 kb band of genomic DNA.

Our studies on the strain-specific nature of V_{κ} Ser gene expression may be compared with studies by Bothwell et al. (58) and Loh et al. (59) on the strain-specific expression of certain V_H regions in the response of C57BL/6 mice to the (4-hydroxyl-3-nitrophenyl)acetyl (NP) hapten. Whereas only one strongly hybridizing V_{κ} Ser-related fragment is detected in DNA of strains that do and do not express the I_B -peptide and $Ef1^a$ markers, those workers detected multiple intensely-hybridizing fragments in strains that do and do not express the NP^b idiomotype. Loh and coworkers showed that in C57BL/6 mice, only one of these V_H genes was used in the response to NP, and that none of the related genes in BALB/c were used, three being pseudogenes, and two containing CDR differences that make them unlikely to be useful for anti-NP antibodies. BALB/c mice use different V_H genes to produce anti-NP antibodies (59). Since the antigen-binding specificity of C.C58 antibodies containing V_{κ} Ser L chains is not known, we are unable to use immunization to help determine whether failure to express V_{κ} Ser-associated phenotypic markers reflects use by nonexpressor strains of a different κ chain group. However, studies of germline V_{κ} Ser-like genes should reveal whether it is at least possible for phenotypically-negative strains to express the I_B -peptide and $Ef1^a$ markers.

Summary

Previous studies (21) have shown that two mouse κ light (L) chain variable (V) region polymorphisms, the I_B -peptide and $Ef1^a$ markers, reflect expression of a characteristic group of V_{κ} regions, called V_{κ} Ser, by some inbred strains and not others. Expression of V_{κ} Ser is controlled by a locus on chromosome 6, the

chromosome that contains the κ locus. To further characterize this V_{κ} group and begin to analyze the basis for its strain-specific expression, full-length complementary DNA (cDNA) copies were produced of L chain mRNA from the M75 myeloma that had been induced in the C.C58 strain of mice, and which produces a V_{κ} Ser L chain. The C.C58 strain is congenic with BALB/cAn, differing in the region of chromosome 6 that controls expression of the V_{κ} polymorphisms and the Lyt-2 and Lyt-3 T cell alloantigens. The complete nucleotide sequence of this cloned cDNA was determined and compared with the nucleotide sequences the most closely related BALB/c myeloma L chains known. Results indicated significant differences throughout the variable region, but particularly toward the 5' portion of the sequence. A probe corresponding to 200 bp of the 5' end of the cloned V_{κ} Ser cDNA was used in Southern hybridizations of restriction digests of liver DNA from a number of inbred, recombinant, and recombinant inbred strains. Under stringent hybridization conditions, one strongly-hybridizing fragment was observed in Bam HI, Hind III, and Eco RI digests, and based on the size of the fragments, strains could be organized into two groups. The presence of strongly hybridizing Bam HI, Hind III, and Eco RI fragments of 3.2, 2.8, and 2.1 kb, respectively, was found to correlate completely with expression by the strain of the I_{B} -peptide and $Ef1^a$ markers. All nonexpressor strains yielded hybridizing fragments of 7.8, 8.4, and 2.8 kb, respectively. Possible explanations for strain-specific expression of V_{κ} Ser-associated phenotypic markers are discussed.

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