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Immunology

Expression and rearrangement of homologous immunoglobulin V_H genes in two mouse strains

(anti-*p*-azophenylarsonate antibodies/idiotype/hybridoma/somatic mutation)

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ABSTRACT **A family of murine anti-*p*-azophenylarsonate (Ars) antibodies share a variable (V) region serologically defined marker, the 36-60 idiotype (Id36-60). Most mouse strains possess five genes highly homologous to the gene encoding the heavy (H) chain V region of antibodies bearing Id36-60 (V_H36-60); however, only one of these genes is ever utilized by hybridomas whose antibodies bind Ars and bear Id36-60. The relevant V_H genes were cloned from A/J and BALB/c mouse DNA libraries. Their DNA sequences were found to differ at only two positions. Southern blot analysis, protein sequence determination, and nucleic acid sequence determination indicate that the above hybridomas utilize the same joining (J_{H3}), diversity (D), and V_H gene segments regardless of BALB/c or A/J strain origin. Despite this virtual identity, BALB/c and A/J mouse strains express quite different serum levels of Id36-60-bearing antibodies when immunized with Ars. The basis of this regulatory process is discussed.**

Antibody genes require rearrangement to form functional variable region genes (V) (1, 2). The κ light (L) chain V region is formed by joining one of several hundred V_L segments with one of four joining (J_L) segments. The heavy (H) chain V region is formed by rearrangement of one of several hundred V_H segments with 1 of 12 estimated diversity (D) segments, which rearranges with one of four J_H segments. These rearrangements bring the assembled V gene into the proximity of a constant region gene (C), whereupon the entire H or L chain is expressed. Antibody diversity is generated by using different combinations of V, D, and J segments, by imprecise site-specific recombination at V_L–J_L or V_H–D–J_H junctions, which can generate hybrid codons or deletions or insertions, by different combinations of H and L chains, and by somatic mutation operating anywhere in the functional V gene (3).

Immunization of certain mouse strains with certain hapten-protein conjugates elicits anti-hapten antibodies, among which a large portion possesses highly related V regions (3–6). These related V regions share structures detected and defined serologically (idiotypes). Such idiotype families often result from expression of one germ-line V_H (and probably one V_L) gene (3–7). Somatic mutations in these germ-line genes accumulate to produce a large family of related antibodies. The hapten *p*-azophenylarsonate (Ars) elicits such responses in A/J mice (8). Approximately 50% of A/J anti-Ars antibodies bear the same idiotype, the major crossreactive idiotype (IdCR) (8, 9). V region sequences of monoclonal antibodies from Ars-immunized mouse hybridomas show that the IdCR proteins are 95% homologous to each other in both V_H and V_L regions (10, 11). The V_H regions of IdCR proteins are derived by somatic mutation and expression from one germ-line gene, the presence of which is correlated with the ability to produce IdCR (7).

Aside from IdCR, other antibody families are present in Ars-immune sera (10–14). One such family represented by the A/J hybridoma 36-60 protein (possessing the 36-60 idiotype, Id36-60), comprises 10–20% of A/J anti-Ars antibodies. IdCR and Id36-60 antibodies are distinct families differing in primary structure, serology, and regulation (14–16). Amino-terminal sequences of Id36-60 hybridoma-derived proteins in A/J mice show extensive homology among both V_H and V_L chains (10, 14). In contrast to IdCR expression, Id36-60 is present in Ars-immune sera from several mouse strains including BALB/c (14). Of particular interest is that the level of Id36-60 expression is a genetic trait such that different strains express different levels of Id36-60 when immunized with Ars.

In order to address the basis of this phenomenon, we examined in detail the expression of the 36-60 idiotype in A/J and BALB/c mice. Therefore, we compared (*i*) Id36-60 abundance in A/J and BALB/c Ars-immune sera, (*ii*) A/J and BALB/c V_HId36-60 hybridoma protein and DNA sequences, and (*iii*) the sequence of the germ-line V_H gene encoding Id36-60 antibodies in each strain.

MATERIALS AND METHODS

Mice. A/J and BALB/c strains were bred in the Massachusetts Institute of Technology animal facility. CBA/Tufts and CBA.Igh^b strains were provided by H. Wortis (Tufts Medical Center, Boston, MA).

Immunizations. Primary immunizations were intraperitoneal injections containing 100 μg of Ars-coupled keyhole limpet hemocyanin in complete Freund's adjuvant. Secondary immunizations 30 days later were identical except that incomplete Freund's adjuvant was used.

Hybridomas. The isolation and characterization from spleen cell SP/2 fusions of the A/J mouse hybridomas whose antibodies bind Ars and possess Id36-60 have been described (14). These hybridomas are 36-54($\mu\kappa$), 31-64($\gamma_{2b}\kappa$), and 36-60($\gamma_{2a}\kappa$). The hybridoma 1210.7($\gamma_1\kappa$) is a BALB/c-derived hybridoma whose antibody weakly binds Ars and possesses Id36-60.

Radioimmunoassays. Sera were assayed for levels of Id36-60, IdCR, and total Ars-binding antibodies as described (14) with the following modification for Id36-60. Anti-Id36-60-coated polyvinyl plastic microtiter plates (Dynatech) were incubated in the presence of ^{125}I-labeled 1210.7 protein and various dilutions of immune serum competitor or monoclonal antibody standards. The dilution resulting in 50% inhibition was compared to the concentration of known standards that cause 50% inhibition. This assay, by definition, only

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Abbreviations: Ars, *p*-azophenylarsonate; kb, kilobase(s); Id36-60, 36-60 idiotype; IdCR, major crossreactive idiotype; H, heavy; L, light; V, variable; D, diversity; J, joining.

monitors Id36-60 antigenic determinants present on both A/J and BALB/c Id36-60-bearing antibodies.

Cloning from Phage Libraries. Partially *Sau*3A-cleaved A/J or BALB/c kidney or 36-60 hybridoma DNA fragments [15–20 kilobases (kb)] ligated with Charon 30 *Bam*HI phage DNA "arms" were packaged (17) to create A/J, BALB/c, and 36-60 libraries (18). Unamplified phage libraries were plated on K802 to eliminate phage endogenous to the packaging system that require *Sup*F. The 3′ J_H hybridization probe was used to screen the 36-60 library for the rearranged V_H36-60 gene, and a phage, S36-2, was found to hybridize to poly(A)$^+$ RNA from 36-60. The isolation of phage clones containing the V_H36-60 germ-line gene is described in the text.

Hybridization Probes. The location of the 3′ J_H probe is shown in Fig. 3 and was subcloned from a plasmid, pRN16, which contains the rearranged V_H gene from hybridoma 36-65 (16). The V_H36-60 probe is a 142-nucleotide fragment from the rearranged V_H36-60 gene in phage S36-2 as denoted in Fig. 3.

Southern Blot Analysis. DNA from mouse kidney or hybridomas (16) was digested, electrophoresed, and transferred to nitrocellulose as described (19). Hybridizations utilizing the 3′ J_H probe involved nick-translation of the entire plasmid and hybridization under aqueous conditions (19). The V_H36-60 142-nucleotide probe was labeled by T4 polymerase replacement synthesis (20) and hybridized under 50% formamide (21) and washed three times for 10 min each at 50°C in 2× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate, pH 7) containing 0.1% $NaDodSO_4$.

DNA and Protein Sequence Determinations. The Maxam and Gilbert (22) sequence determination strategy for the V_H36-60 genes is shown in Fig. 3*C*. The V_H protein sequence of hybridoma 1210.7 was determined by previously described methods (15, 23).

RESULTS

Expression and Characterization of Id36-60 in A/J and BALB/c Mice. We monitored Id36-60, IdCR, and total anti-Ars antibody concentrations in pooled mouse serum during primary and secondary immune responses in groups of BALB/c and A/J mice. Id36-60 dominated anti-Ars responses in BALB/c but not in A/J mice where IdCR dominated (Fig. 1). During primary responses in A/J mice, IdCR appeared first and by 30 days reached a concentration of 1.5 mg/ml, whereas Id36-60 reached only 40 μg/ml. The secondary response in A/J mice induced IdCR antibodies to a concentration of 5 mg/ml (about 50% of the total anti-Ars response) and Id36-60 antibodies to only 1.6 mg/ml (15% of the anti-Ars response). BALB/c mice lack the genetic capability to express IdCR (7) but expressed Id36-60 at levels up to 50% of the total secondary anti-Ars response. In BALB/c primary and secondary responses, Id36-60 reached concentrations of 170 μg/ml and 5 mg/ml, respectively.

The experiments summarized above used pooled sera and, thereby, represent the average idiotype response. To determine if the "average" response was present in every individual, amounts of Id36-60 relative to IdCR were determined in secondary responses of 20 individual A/J and 20 BALB/c mice. In each A/J mouse, the quantity of IdCR exceeded that of Id36-60, although relative amounts were extremely variable; Id36-60 ranged from 0.5% to 87% of the amount of IdCR (average, 17%). Among individual BALB/c mice, concentrations of Id36-60 antibodies were also quite variable (0.5–5.0 mg/ml).

Id36-60 Hybridomas Derived from BALB/c and A/J Mice Possess a Common V_H Gene Rearrangement. The V_H amino-terminal sequences of several Id36-60 proteins and the complete V_H sequence of 36-60 (A/J mice) have been described (10, 11, 14, 15). The protein sequence of 1210.7 (BALB/c mice) is described here (see Fig. 4). All V_H36-60 regions are highly homologous. Hybridoma proteins 36-60 and 1210.7 differ by only four amino acids through the entire V_H region. They share highly homologous V_H and identical D and J_{H3} segments. The protein sequences agree with Southern blot data in which all Id36-60 hybridoma DNAs show a 1.9-kb *Eco*RI fragment containing the rearranged V_H gene (Fig. 2*A*) as detected with a 3′ J_H probe. Because the *Eco*RI site 3′ of J_{H4} is known (24), the 1.9-kb length is enough to include a *V/D* rearrangement to J_{H3} or J_{H4}, but not to J_{H2}. Southern blots (not shown) with the 3′ J_H probe and *Hin*dIII (which cuts between J_{H3} and J_{H4}) showed that J_{H3} is used in all Id36-60 hybridomas. The 1.9-kb size of the *Eco*RI piece and the use of J_{H3} indicates that the 5′ *Eco*RI site is within the V_H segment. The V_H36-60 probe with *Hin*dIII digests also

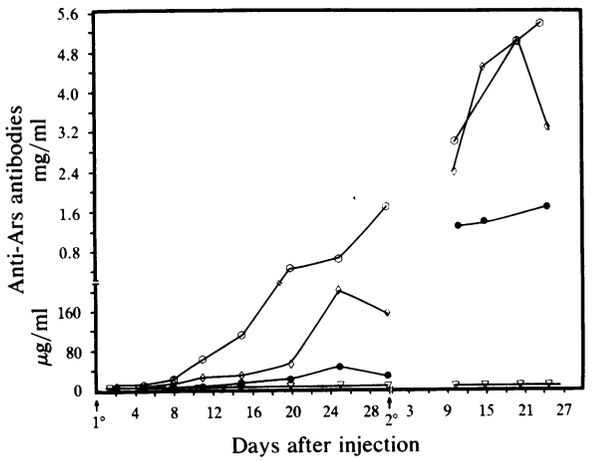

FIG. 1. The appearance of Id36-60- and IdCR-bearing anti-Ars antibodies in A/J and BALB/c mouse sera during primary (1°) and secondary (2°) Ars immune responses. Twelve mice from each strain divided into four groups of three mice were immunized, and sera from each group were pooled and analyzed by radioimmunoassay. Each group was bled at a different time point. ▽, IdCR in BALB/c; ○, IdCR in A/J; ◇, Id36-60 in BALB/c; ●, Id36-60 in A/J mice.

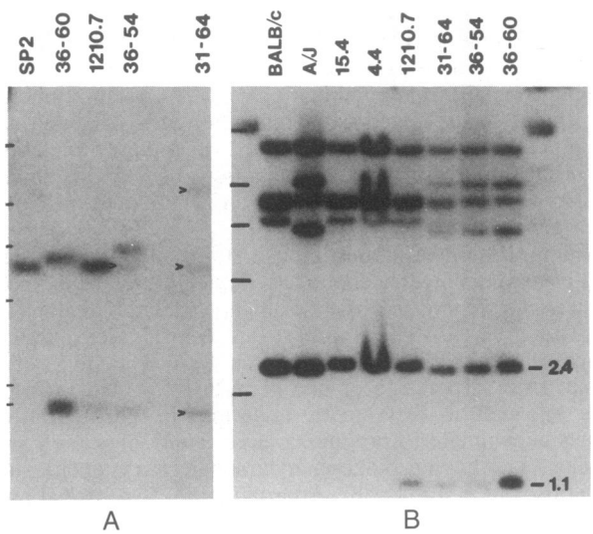

FIG. 2. Southern blot analysis of V_H rearrangement in Id36-60-bearing hybridoma DNAs. DNAs from the indicated hybridomas were digested with *Eco*RI (*A*) or *Hin*dIII (*B*), electrophoresed, blotted, and hybridized with the 3′ J_H probe. *Hin*dIII-cleaved λ phage DNA fragments were size markers. Weak bands in *A* are denoted by carats. The germ-line and rearranged V_H36-60 bands (2.4 and 1.1 kb, respectively) are noted in *B*. The BALB/c hybridomas Max 4.4 and 15.4 (supplied by C. Henry) no longer secrete Id36-60 and also show an absence of rearranged V_H36-60.

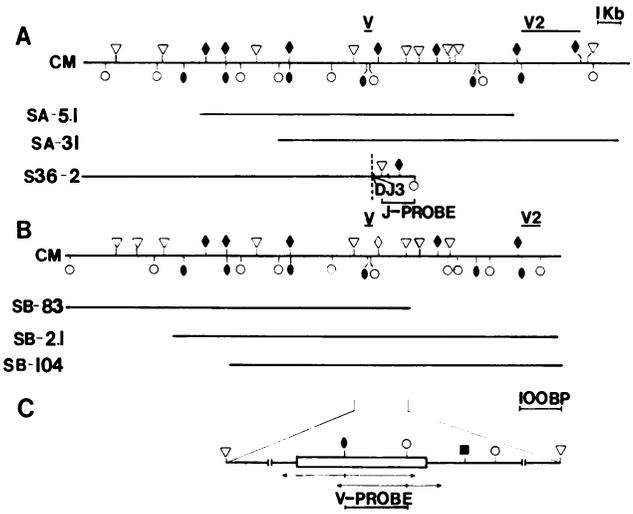

FIG. 3. Restriction site maps of A/J and BALB/c germ-line V_H36-60 genes and the rearranged V_H36-60 gene from A/J hybridoma 36-60. These genes were isolated from phage libraries. The individual restriction maps are aligned by homologous DNA regions. The composite germ-line map (CM) is shown above each set of phage inserts. (*A*) Inserts of two A/J germ-line phage (SA-5.1 and SA-31) and of the rearranged V_H36-60 phage S36-2 (note: all phage possess the 1.8-kb *Eco*RI piece containing V_H36-60). (*B*) Inserts of three BALB/c V_H36-60 germ-line phage (SB-83, SB-2.1, SB-104; all possess the 1.8-kb piece). (*C*) General sequence determination strategy for V_H36-60. ●, *Bam*HI; ○, *Eco*RI; ▽, *Hin*dIII; ■, *Rsa* I; and ◆, *Xba* I. 5′ → 3′ is left to right.

detected identical rearrangements in all Id36-60 hybridoma DNAs (Fig. 2*B*). These data suggest the use of one germ-line V_H, *D*, and J_{H3} segment producing all Id36-60 proteins.

Cloning the Rearranged and Germ-line V_H36-60 Genes. In order to examine the V_H gene expressed in Id36-60 hybridomas, we cloned the rearranged V_H gene from the hybridoma 36-60. The rearranged V_H36-60 gene was isolated from a *Sau*3A library of 36-60 DNA. The restriction map of phage S36-2, containing the rearranged V_H36-60 gene, is shown in Fig. 3*A*. The 12.5 kb of 5′ DNA should be shared with the germ-line V_H36-60 gene. The V_H36-60 gene resides within a 1.8-kb *Eco*RI fragment, which is identical whether rearranged or not and is useful for identifying the germ-line V_H36-60 gene. A 4.6-kb *Xba* fragment containing the rearranged V_H36-60 gene was subcloned, and the V_H36-60 sequence was found to agree with the protein sequence and to confirm usage of J_{H3} (Figs. 3*C* and 4). No polymorphisms are present in J_{H3} and J_{H2} (16) from A/J mice compared to BALB/c mice (24).

A 142-nucleotide internal *Bam*HI–*Eco*RI fragment was subcloned from the rearranged V_H36-60 (Fig. 3*C*) and was used as a hybridization probe. *Sau*3A libraries from A/J and BALB/c kidney DNA were produced and screened for the most homologous plaques in order to clone the Id36-60 germ-line genes. Eight BALB/c and seven A/J phage were selected and mapped for restriction sites. Three of the BALB/c and two of the A/J clones possessed the 1.8-kb V_H36-60-containing *Eco*RI piece. Furthermore, these phage contained the correct *Hin*dIII site 5′ of V_H36-60. This is shown in Fig. 2*B*, where *Hin*dIII-digested Id36-60 hybridoma DNAs display identical 1.1-kb rearranged V_H36-60 bands. Because the location of the 3′ *Hin*dIII site is known (between J_{H3} and J_{H4}), one can determine the 5′ site location in the germ-line clones. The cloned germ-line V_H genes also possess internal *Bam*HI sites and share extensive 5′ restriction site identity with the rearranged V_H36-60. These clones represent only one of five genes of the 36-60 V_H family (see below). The

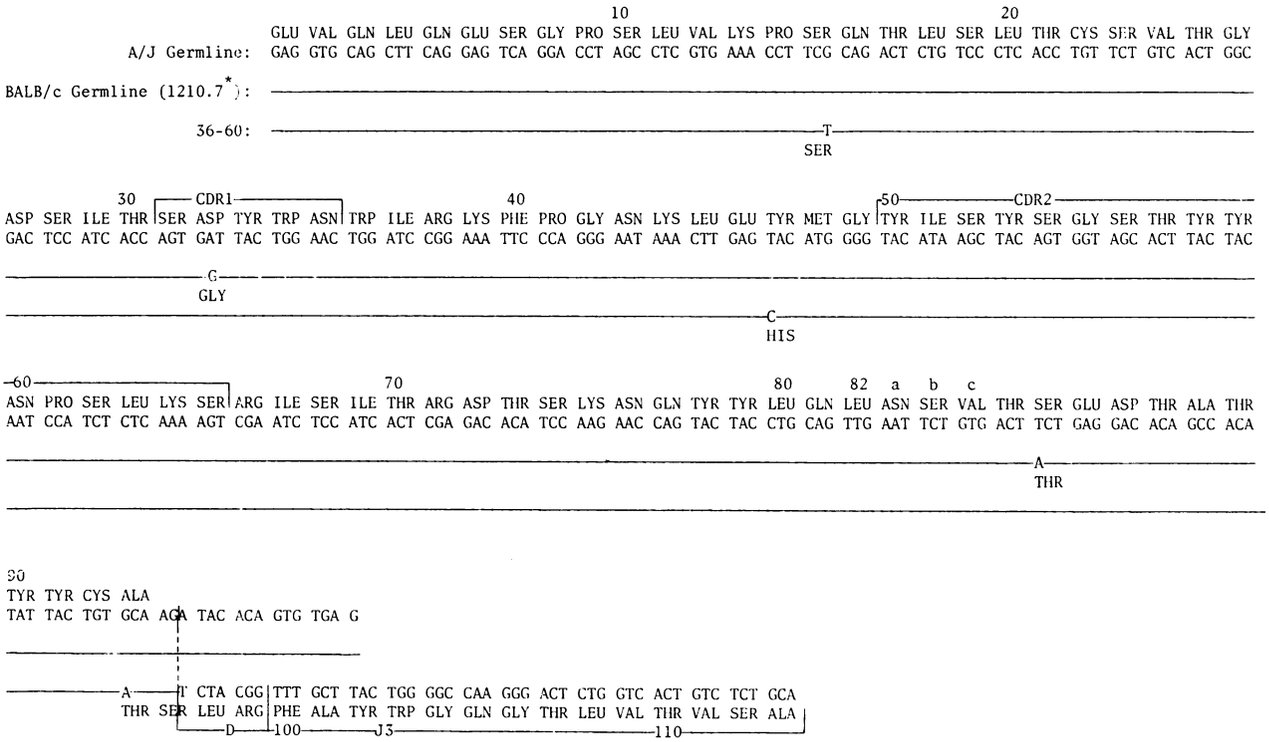

FIG. 4. DNA sequences of A/J and BALB/c germ-line and rearranged V_H36-60. The 2.4-kb *Hin*dIII germ-line fragment was subcloned from A/J phage SA-5.1 and BALB/c phage SB-2.1. The 4.6-kb *Xba* I rearranged fragment was subcloned from the 36-60 hybridoma-derived phage S36-2. CDR, complementarity determining regions; the numbering is according to Kabat *et al.* (1). *, BALB/c germ-line sequence predicts a translated V_H polypeptide that precisely matches the amino acid sequence of the BALB/c hybridoma protein 1210.7. The DNA sequence of 1210.7 was not determined and could contain silent mutations. The A/J germ-line sequence is used as the prototype, with its identity denoted by a horizontal line. Nucleotide differences from this prototype are shown along with any resulting amino acid differences. The small area (Fig. 4*C*) with its sequence determined in one direction was unambiguous; its sequence was redetermined twice.

cloned DNAs from A/J (Fig. 3*A*) and BALB/c (Fig. 3*B*) mice overlap to form composite maps of 24.5- and 22.5-kb length, respectively. The significant homology between these maps is consistent with these regions being allelic. The first polymorphism 5′ of V_H36-60 is a *Hin*dIII site 10.2 kb upstream in BALB/c DNA (Fig. 3*B*). The 3′ area shows greater polymorphism starting with an *Eco*RI site 3.4 kb downstream (Fig. 3*B*). Within the cloned DNA of both A/J and BALB/c mice, a second homologous V region about 7 kb 3′ of V_H36-60 is present.

*Hin*dIII fragments of 2.4 kb were subcloned, and the sequence of the V_H gene was determined (Figs. 3*C* and 4) from A/J and BALB/c V_H36-60 germ-line phage inserts. There are only two nucleotide disparities between the two strains, both resulting in codon differences; a nonconservative Asp/Gly difference at position 32 in CDR1 and a conservative Ser/Thr change at position 84. The difference at position 32 is of interest because two A/J hybridoma proteins, 31-64 and 36-54, have mutations there resulting in a change from Asp to Asn. Because μ H chains often lack somatic mutations, the mutation in 36-54 is noteworthy (3). Both 36-60 and 31-64 have nonconservative changes at position 47. The rearranged V_H36-60 gene from the hybridoma 36-60 possesses only three mutations (Fig. 4): a silent mutation at position 15, a Tyr/His change at 47, and an Ala/Thr change at 94. The BALB/c germ-line DNA sequence agrees entirely with the BALB/c hybridoma 1210.7 amino acid sequence (Fig. 4). The V_H36-60 germ-line and J_{H3} (24) sequences allow assignment of the *D* region (derived from the $D_{FL16.1}$ gene) (2). The *D* region encodes only two amino acids at positions 98 and 99 (Leu-Arg) in A/J protein 36-60 and BALB/c protein 1210.7.

The V_H36-60 Gene Family. We examined the distribution and number of restriction fragments containing V_H36-60 homologous DNA among mouse strains. Kidney DNA from nine strains was digested with *Hin*dIII (Fig. 5*A*) or *Eco*RI (Fig. 5*B*) and analyzed by Southern blots with the V_H36-60 probe. In A/J and BALB/c kidney DNA digests, four bands of equal intensity and a fifth strong band, the V_H36-60 germ-line 2.4-kb *Hin*dIII (Fig. 5*A*) band, were detected. A/J and BALB/c DNA digests share three bands and have two polymorphic bands (Fig. 5*A*). Not all strains show five bands of equal intensity; AKR, CBA, and NZB show the intense 2.4-kb band, two intermediate intensity bands, and two light bands (Fig. 5*A*). The band pattern in Fig. 5*A* may be used to assign mice to related groups: AKR, CBA, and NZB mice share identical patterns; A/J and C.AL-20 (a congenic mouse strain possessing the AL/N *Igh* locus on a BALB/c background) are identical as expected; and B10.A and CBA.*Igh*b, which share allotypes, are almost identical. DBA/2 and BALB/c are unique. Allotype markers do not always correlate with the V_H36-60 pattern because different *Igh-1* markers [as in AKR(Igh^d), NZB(Igh^e), and CBA(Igh^j)] may share patterns, whereas strains that share *Igh-1* markers [B10.A(Igh^b) and CBA.Igh^b or AKR(Igh^d) and C.AL-20(Igh^d)] may have different patterns.

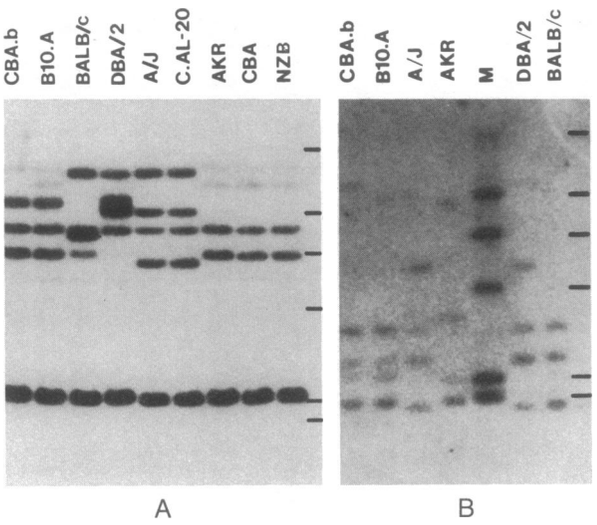

FIG. 5. Polymorphisms in the V_H36-60 gene family. Kidney DNA from the indicated strains was digested with *Hin*dIII (*A*) or *Eco*RI (*B*) and analyzed by Southern blots using the V_H36-60 probe. Markers are 5′-labeled *Hin*dIII-cleaved phage λ DNA fragments. An additional band of 1.3 kb is present in blots of A/J and BALB/c *Eco*RI DNA digests when hybridization was stronger.

The *Eco*RI digests (Fig. 5*B*) show similar sets of V_H36-60 band patterns. The 1.8-kb germ-line gene shows an interesting polymorphism; in A/J, BALB/c, and DBA/2 DNA digests, it appears as a 1.8-kb piece, whereas in CBA.Igh^b, B10.A, and AKR DNA digests, it migrates at 1.9 kb. This correlates with the ability to express Id36-60 because A/J, BALB/c, C.AL-20, and DBA/2 strains express Id36-60, whereas B10.A does not (refs. 14 and 25; unpublished data). (AKR and CBA.Igh^b have not been tested for Id36-60.) Therefore, the appearance of a 1.8-kb V_H36-60 band is a more consistent marker for Id36-60 production than is allotype (26).

DISCUSSION

In this study we examined the relationship between the V_H36-60 germ-line genes and the expression of the 36-60 idiotype in two strains of mice. We found that, despite the use of highly homologous V_H segments and identical *D* and J_{H3} segments, different levels of Id36-60 are expressed in these two strains. Id36-60 is the predominant idiotype among anti-Ars antibodies in BALB/c mice. In A/J mice, IdCR is the predominant idiotype family (8), whereas Id36-60 represents a relatively small portion of anti-Ars antibodies. IdCR, Id36-60, and the ratio Id36-60/IdCR fluctuate widely among individual A/J immune sera. This may reflect a stochastic process occurring during the generation and expansion of precursor B cells.

Studies show that somatic mutation of the germ-line V_H gene may lead to increased hapten affinity of the encoded antibody (27, 28). The association constants for affinity for Ars of three A/J Id36-60 hybridoma proteins (36-60, 31-64, and 36-54) have been measured (27). These constants are similar to each other and to the affinities of IdCR proteins. However, the Ars affinity of the BALB/c Id36-60 hybridoma protein 1210.7 is very weak. The V_H region of 1210.7 protein is directly encoded without mutation by a BALB/c germ-line V_H gene. If the A/J germ-line V_H36-60 gene encodes a protein with weak Ars affinity (resembling the highly homologous germ line-encoded BALB/c antibody 1210.7), the Id36-60 precursor B cell may be required to undergo several mutations before acquiring an Ars affinity that is adequate to trigger proliferation. Because the germ-line V_H gene for IdCR encodes the H chain of antibodies that bind Ars strongly (7, 27), IdCR precursor B cells may be triggered to proliferate without mutation. The kinetics of appearance of ID36-60 and IdCR in A/J mice (Fig. 1) are consistent with this hypothesis. Therefore, competition for antigen binding may select for the IdCR that dominates in A/J mice, whereas Id36-60 dominates in BALB/c mice, where no such competition occurs.

This hypothesis predicts that Id36-60 hybridoma proteins would bear more mutations than would IdCR proteins. In fact, Id36-60 proteins show little variability (23) as compared with most IdCR proteins (10, 11). This lack of variability may be accounted for by assuming that mutations in the weakly Ars-binding V_H36-60 germ-line protein would as likely eliminate Ars-binding ability as enhance it. In the phos-

phorylcholine system, single mutations within the H chain V region markedly affect antigen binding (29, 30). Careful selection of mutations in V_H36-60 might be necessary if enhanced Ars binding were to result. The appearance of asparagine twice at position 32 and the variability at position 47 in independent Id36-60 A/J hybridoma proteins may represent selection of certain mutations at certain positions. The presence of private idiotypes (serological markers on individual antibodies) expressed in every individual of a mouse strain (31) may be another example of such recurrent somatic mutations. Strong selection by antigen or possibly gene conversion may be responsible for generating these recurrent somatic variants (32, 33). The sequences of the other V_H36-60 homologous genes (Fig. 5) should provide evidence pertaining to the latter possibility. The L chain, which has not been considered, may contribute relatively little to the enhancement of Ars binding because preliminary data indicate that the V regions of A/J and BALB/c hybridoma protein L chains may differ consistently by only one amino acid.

It is noteworthy that the same D and J_H segments are used in Id36-60 hybridoma proteins. This situation might be required for antigen binding or the presence of idiotypy. Because the hybridomas were selected for both Id36-60 and Ars binding, utilization of identical V_H, D, and J_{H3} segments is not too surprising. Ars-nonbinding IdCR hybridoma proteins exist that differ from Ars-binding IdCR proteins in utilizing different J_H segments (23). Phosphorylcholine-binding hybridoma proteins, related to the T15 idiotype, from C57BL, CBA, and BALB/c strains, share V_H, V_L and J_{H1} regions but often use different D regions (34). Because these proteins all bind phosphorylcholine, it appears that the D region need not be conserved for antigen binding ability in this system.

The mechanisms that generate somatic variability also could contribute to variability among germ-line genes. Gene conversion may represent such a mechanism for germ-line V_H genes (35, 36). The difference at codon 32 between the A/J and BALB/c V_H36-60 germ-line genes can be accounted for by a gene conversion event whereby a codon (GGT) from an A/J V_H36-60 family member replaces the A/J V_H36-60 germ-line codon (GAT). This replacement matches the BALB/c germ-line V_H36-60 sequence. The V_H36-60 homologous gene within the A/J 2.7-kb *Eco*RI band (Fig. 5*B*) may represent such a family member because partial sequence determination reveals the GGT codon at position 32.

The level of expression of a given V_H or V_L gene is regulated. In the case of IdCR, the level of idiotype expression is controlled by the presence or absence of a structural gene (16). For Id36-60, however, the level of expression is apparently genetically controlled. Id36-60 is a minor idiotype in A/J mice and the predominant idiotype in BALB/c. Several mechanisms, aside from the above affinity argument, may contribute to control the expression of Id36-60. The frequency of formation of an effective *V–D–J* joining and pairing with an appropriate L chain (37) may affect the number of precursor B cells capable of expressing a given idiotype (38). Furthermore, cellular regulation by helper (39) and suppressor (40) T cells must be considered. Gene dosage does not seem to be a factor because IdCR (7) and Id36-60 families appear to be derived from single genes.

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