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Immunology

Murine T-cell receptor mutants with deletions of β-chain variable region genes

(cDNA clones/gene expression/spleen)

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ABSTRACT **Genomic Southern blots of DNA from eight strains of mice were examined for restriction fragment length polymorphisms in their loci encoding the variable region of the T-cell receptor β chain (V_β), using 16 different V_β-specific probes. Mouse strains BALB/c, C57BL/6, C3H, and PL were identical, while strains SJL, C57BR, C57L, and SWR shared several polymorphisms with respect to the other four strains. In addition, SJL, C57L, C57BR, and SWR DNAs were missing 50% of the hybridizing bands visualized in BALB/c DNA. A cDNA library from concanavalin A-stimulated SJL spleen blasts was constructed and examined for V_β gene usage. Ten genes were found to account for all V_β-containing clones isolated, including three newly identified V_β genes. All 10 of these genes were found to be present in BALB/c mice. We conclude that SJL, C57L, C57BR, and SWR mice represent V_β deletion mutants of the BALB/c genotype.**

The β chain of the murine T cell receptor (TCR) is composed of an amino-terminal variable region (V_β) and carboxyl-terminal constant region (C_β) (1). A functional V_β is produced by a series of somatic DNA rearrangements between germ-line variable (V_β), diversity (D_β), and joining (J_β) gene segments (2), similar to the DNA rearrangements required to produce a functional immunoglobulin heavy chain variable region (V_H) (3). There exist many germline immunoglobulin V_H genes (100–300), which have been grouped by cross-hybridization into subfamilies of 5–40 genes (4); in contrast, it appears that there are relatively few expressed TCR V_β genes (<30) (5, 6), most of which belong to single-element subfamilies (5–7). Murine V_H genes show significant interstrain polymorphism in several V_H subfamilies examined (8, 9), while V_β alleles show <1% polymorphism between strains (5).

Serologically defined constant region (C_H) allotypes exist for immunoglobulin heavy chain that have been correlated with defined V_H restriction fragment polymorphisms on genomic Southern blots (10). At present, two serologic reagents have been described that are believed to define a murine TCR β-chain allotype (11, 12). We examined eight inbred strains of mice, four of which react with these reagents and four of which do not, for V_β-associated restriction fragment length polymorphisms (RFLPs) on genomic Southern blots. We find that the lack of reactivity of T cells from certain inbred mouse strains with these reagents correlates with a set of V_β polymorphisms that includes a germ-line deletion of ≈50% of known V_β genes.

MATERIALS AND METHODS

Construction and Screening of the SJL cDNA Library. Total cellular RNA was prepared from 10^9 SJL spleen Con A-stimulated blasts by the guanidinium isothiocyanate method (13). Poly(A)$^+$ RNA was purified (ref. 14, p. 197) and a cDNA library was constructed in the λgt10 cloning vector as described (15), with minor modifications (16). This library was screened with a murine C_β-containing cDNA clone (kindly provided by T. Mak) as described (ref. 14, p. 320). C_β-positive phage were screened with probes specific for V_β1, -2, -3, -4, -6, -7, and -10; cDNAs that were positive with the C_β probe but negative with existing V_β probes were subcloned in pUC12 and sequenced by the method of Maxam and Gilbert (17).

Southern Blot Analysis. High molecular weight genomic DNA was prepared from the livers of BALB/c, C57BL/6, C3H, PL, SJL, C57BR, C57L, and SWR mice (ref. 14, p. 280). DNAs were digested to completion with *Eco*RI, *Msp* I, *Pvu* II, and *Sac* I, separated on 0.8% agarose gels, and transferred to nitrocellulose filters (18). DNA on the filters was hybridized to nick-translated DNA (specific activity of 1–3 × 10^8 cpm/μg) at 68°C for >12 hr under standard conditions (ref. 14, p. 324). Filters were washed in 0.30 M NaCl/0.03 M sodium citrate at 68°C and autoradiographed.

RESULTS

SJL V_β Genes Missing. We had reported earlier that the SJL mouse is missing many of the TCR V_β genes present in other inbred strains, as assessed by Southern blot analysis (5). This analysis has been extended to include 16 different V_β-specific probes, representing every published V_β gene segment, as well as three additional V_β genes presented here. These probes were hybridized to Southern blots of BALB/c, C57BL/6, C3H, PL, and SJL liver DNA digested with *Eco*RI, *Msp* I, *Pvu* II, and *Sac* I, as summarized in Table 1. The 16 V_β probes hybridized to 20 bands in BALB/c, C57BL/6, C3H, and PL DNA, while only 10 bands were visualized in SJL DNA. Thus, ≈50% of identified V_β genes are missing from the SJL genome.

No RFLPs were observed in BALB/c, C57BL/6, C3H, or PL mice by using the four indicated restriction endonucleases with any of the V_β probes. Of the 10 bands visualized in SJL DNA, those hybridizing to probes V_β2, V_β4, V_β7, V_β14, V_β15, and V_β16 were identical to the bands seen by using DNA from the other four strains. However, SJL DNA did show RFLPs when probes V_β1, V_β3, V_β6, and V_β10 were used. V_β1 showed RFLPs with three enzymes (*Msp* I, *Pvu* II, and *Sac* I), V_β3 showed RFLPs with two enzymes (*Eco*RI and *Msp* I), V_β6 showed an RFLP with 1 enzyme (*Msp* I), and V_β10 showed RFLPs with all four enzymes.

V_β Gene Expression in SJL Spleen. As suggested earlier (5), the absence of certain V_β genes in the SJL genome can be accounted for in two ways: (*i*) SJL has suffered a deletion involving ≈50% of known V_β genes, or (*ii*) SJL possesses a

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Abbreviations: TCR, T-cell antigen receptor; V, variable; D, diversity; J, joining; C, constant; RFLP, restriction fragment length polymorphism.

Table 1. Summary of genomic Southern analysis

V_β probe*	Examples and refs.	No. bands visualized: BALB/c, C57BL/6, C3H, PL	No. bands visualized: SJL
V_β1	86T1 (1), V_β11 (5)	1	1†
V_β2	E1 (7), V_β6 (5)	1	1
V_β3	2B4 (2), 3H.25 (19)	1	1†
V_β4	TB3 (6), V_β9 (5)	1	1
V_β5.1	TB21 (6), V_β8 (5)	3	0
V_β6	LB2 (7), V_β1 (5)	1	1†
V_β7	pHDS11 (20)	1	1
V_β8.2‡	TB2 (6), V_β4 (5)	3	0
V_β9	V_β2 (5)	1	0
V_β10	V_β3 (5)	1	1†
V_β11	V_β5 (5), AK1 (21)	1	0
V_β12	V_β7 (5)	1	0
V_β13	V_β10 (5)	1	0
V_β14	SJL 33 (this work)	1	1
V_β15	SJL 73 (this work)	1	1
V_β16	SJL 4 (this work)	1	1

*V_β nomenclature follows that of Barth *et al.* (6).
†RFLPs noted with respect to the other four strains.
‡C5 three-member family (7).

complete set of V_β genes, many of which are distinct from those present in the other inbred strains. If the latter hypothesis is correct, it should be possible to identify V_β genes expressed in SJL T cells that are absent from BALB/c or C57BL/6 DNA. We therefore screened an unamplified cDNA library from Con A-stimulated SJL spleen blasts with a C_β specific probe in an attempt to identify V_β gene segments expressed in SJL that had not already been identified from BALB/c or C57BL/6. We isolated 61 C_β-positive clones from 75,000 screened; these 61 clones were probed with the seven V_βs already known to exist in SJL (V_β1, -2, -3, -4, -6, -7, and -10) (5, 6). Clones not hybridizing to known V_βs were subcloned and their DNA sequence was determined. Of the 61 C_β-containing clones, 36 hybridized to the seven V_β probes. Of the remaining 25 clones, 8 proved to be too short to contain V regions, while 9 originated from unspliced or aberrantly spliced transcripts and therefore did not contain V_β sequences (sequence data not shown). One clone was identified as a "*D–J*" transcript (22), in which D_β2.1 joined to J_β2.7 with no V_β sequence involved, while another clone involved an unrearranged J_β2.3 spliced correctly to C_β2 (sequence data not shown) (23). Nineteen clones, therefore, were C_β-positive but did not contain V_β sequences. The remaining 6 clones did contain V_β segments and represented the usage of three previously unknown V_β gene segments (designated V_β14, -15, and -16); the DNA sequences of these three V_β genes are presented in Fig. 1. The identities of all clones obtained from the SJL spleen library are summarized in Table 2.

The V regions of these three clones were used to probe Southern blots of BALB/c, C57BL/6, C3H, PL, and SJL DNA digested with *Eco*RI, *Msp* I, *Pvu* II, and *Sac* I; the blot probed with V_β16 is presented in Fig. 2. Probes specific for V_β14, V_β15, and V_β16 each hybridized to single bands on genomic Southern blots that were present in all five strains and nonpolymorphic with all four enzymes. In addition, V_β14, -15, and -16 were used to probe Southern blots of *Eco*RI-digested DNA from an expanded series of mice, including two wild mice. As seen in Fig. 2, these genes are present and nonpolymorphic in all mice tested, including the wild mice.

In summary, of 61 C_β-containing cDNAs examined, 42 contained a V_β gene segment. These 42 independent cDNAs

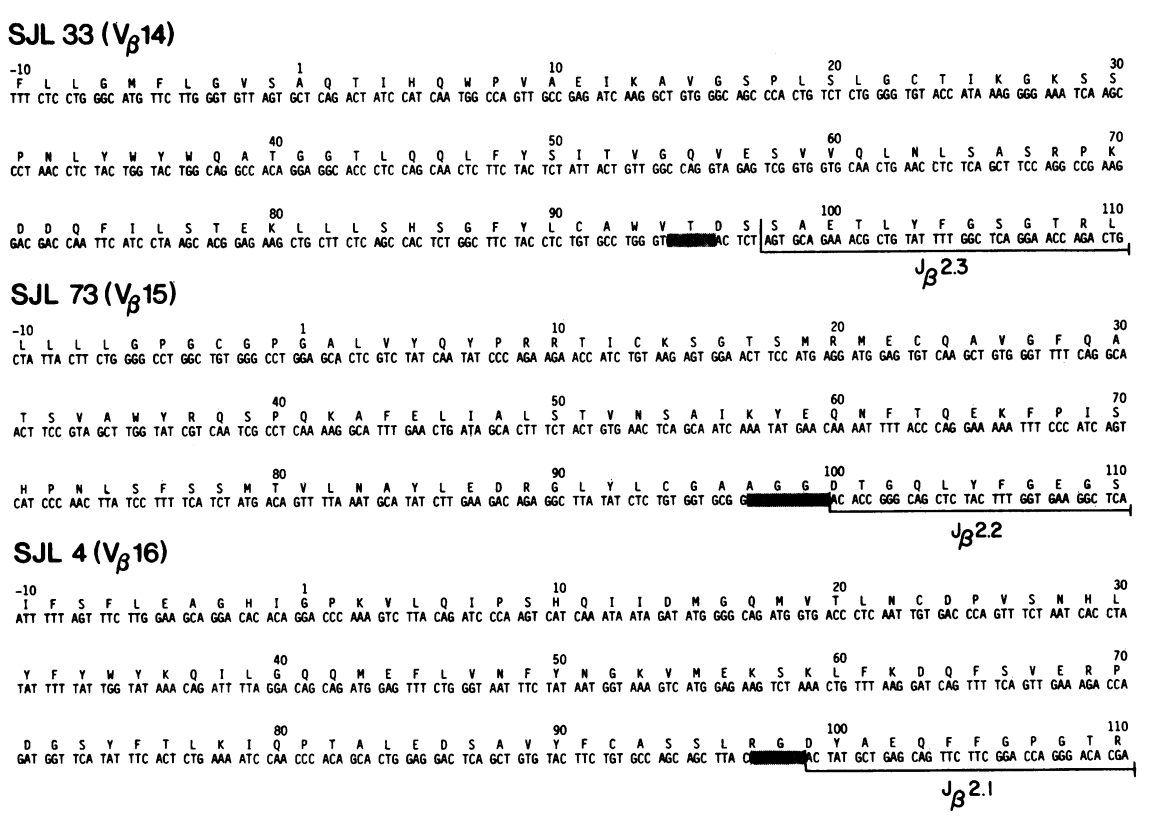

FIG. 1. Nucleotide and amino acid sequences of the *VDJ* regions from three C_β-positive isolates from a cDNA library of Con A-stimulated SJL spleen blasts. Codon numbering following that of Patten *et al.* (7). Sequence contributed from germ-line D_β1.1 or D_β2.1 elements are highlighted, while J_β gene segments are underlined. Nucleotides between D_β and J_β segments are proposed N region insertions.

Table 2. Summary of SJL spleen cDNA clones

Identities of 61 C_β-containing clones*	No. of isolates of this type
$V_\beta 1$	2
$V_\beta 2$	5
$V_\beta 3$	5
$V_\beta 4$	2
$V_\beta 6$	11
$V_\beta 7$	7
$V_\beta 10$	4
$V_\beta 14$	1
$V_\beta 15$	3
$V_\beta 16$	2
Other†	19

*V_β nomenclature as in Table 1.
†C_β-positive clones that did not contain a V_β gene segment.

represent the usage of only 10 different V_β genes, all of which are present in BALB/c, C57BL/6, C3H, PL, and SJL mice. No expressed V_β genes were found in SJL that were not present in the other inbred strains. Thus, we conclude that the SJL mouse is a V_β deletion mutant and probably does not contain V_β genes absent in other strains.

SJL V_β, J_β, and C_β Polymorphisms. It has been reported that SJL DNA shows RFLPs in both the V_β (5) and C_β (24) regions, while other common strains are indistinguishable. To assess TCR β-chain-associated polymorphism (possible TCR allotypes), SJL sequences obtained from our spleen cDNA library were compared with published BALB/c and C57BL/6 sequences. The sequences of $V_\beta 3$/2B4, $V_\beta 7$/pHDS11, $J_\beta 1.2$, -2.1, -2.2, -2.3, -2.4, -2.5, -2.7, and $C_\beta 1$ have been compared. The SJL $V_\beta 3$ allele and the published C57BL/6 2B4 sequence (2) differ by 2 bases over 280 bases compared. Similarly, the SJL $V_\beta 7$ allele and the published BALB.B pHDS11 sequence (20) differ by only a single base over 300 bases compared. We have sequenced a $V_\beta 7$ gene from a cDNA library of Con A-stimulated C57BL/6 spleen blasts and found it to be identical to the SJL $V_\beta 7$ gene (data not shown). SJL V_β genes therefore seem to be virtually identical to their allelic counterparts in mice lacking the V_β deletion event.

The sequence of seven different J_β segments have been obtained from our SJL cDNA clones. We find the SJL $J_\beta 1.2$, -2.2, -2.3, -2.4, and -2.5 gene segments to be identical to the published C57BL/6 sequence (2, 25), while $J_\beta 2.1$ has a single base change and $J_\beta 2.7$ has two base changes with respect to the published C57BL/6 sequence (2). All three of these changes are silent at the amino acid level. The SJL $C_\beta 1$ and published BALB/c $C_\beta 1$ alleles (1) differ by 2 bases over 518 compared, both changes resulting in replacement at the amino acid level (data not shown).

While it is clear that SJL has a significant V_β deletion and displays several RFLPs, these genes are nevertheless very similar to their alleles in BALB/c or C57BL/6, showing ≈0.5% difference between strains over 1600 bases of V_β, J_β, and C_β DNA sequence compared. This degree of divergence is similar to that described earlier between V_β genes isolated from BALB/c and C57BL/6 (5).

SJL-Type V_β Deletion in Other Mouse Strains. We had suggested earlier that the TCR allotypic reagent KJ16-133 described by Marrack and co-workers (11) was directed against a determinant specific to a subset of V_β genes missing in the SJL mouse (5). If this is true, other strains of mice that do not react with KJ16-133 should also have the same V_β deletion event described for the SJL mouse. To address this question, Southern blots of liver DNA from C57L, C57BR, and SWR mice (24) were probed with our 16 V_β clones. Representative blots are shown in Fig. 3. The hybridization patterns of C57L, C57BR, and SWR DNAs are identical to the pattern of SJL DNA; probes for $V_\beta 5$, -8, -9, -11, -12, and -13 gave no detectable hybridization, while probes specific for $V_\beta 1$, -2, -3, -4, -6, -7, -10, -14, -15, and -16 each hybridized to single bands. RFLPs in SJL noted earlier with probes $V_\beta 1$, -3, -6, and -10 were also found in C57L, C57BR, and SWR. Therefore both RFLPs and the absence of the same set of V_β genes indicate that SJL, C57L, C57BR, and SWR mice are similar at their V_β loci, having apparently inherited a part of chromosome 6 (26) that has undergone a deletion of ≈50% of known V_β genes.

Interestingly, while these strains all share identity at the V_β loci, they are different from each other at other loci involved in the TCR. Others have noted (24) and we have confirmed (Fig. 3) that RFLP at the $C_\beta 1$ locus classifies C57BR and C57L with C57BL/6, BALB/c, C3H, and PL strains, while SJL and SWR share an *Eco*RI RFLP. Also, using a V_α probe specific for the V_α segment used in CTL clone F3 (sequence data to be presented elsewhere), we note that RFLPs group C57BR, C57L, and C57BL/6 together while other strains show different patterns (Fig. 3). In contrast, the *Eco*RI, *Msp* I, *Pvu* II, and *Sac* I restriction fragment patterns visualized

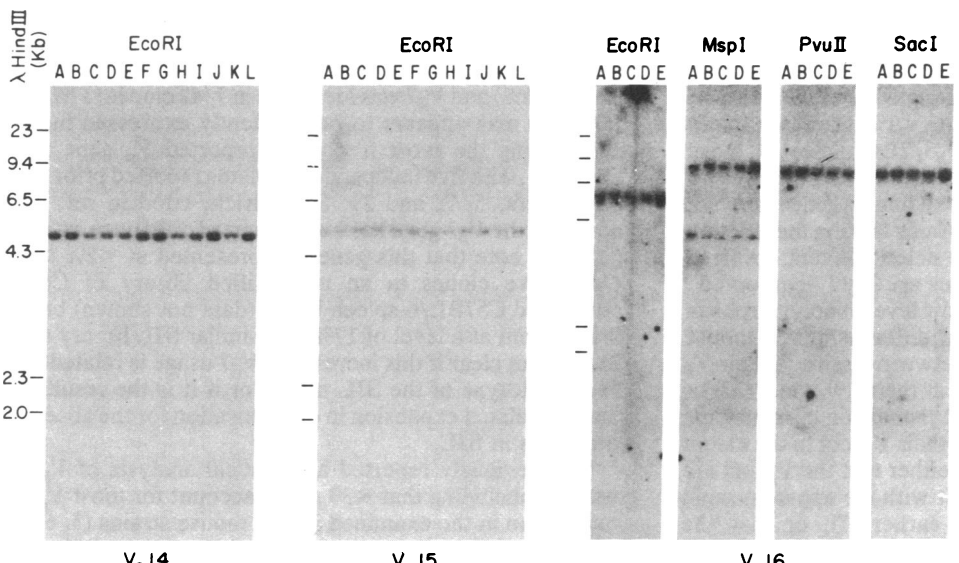

FIG. 2. Genomic Southern blot (18) of liver DNA from inbred and wild mice. DNAs were digested with the indicated restriction endonucleases and hybridized to V_β-specific probes. Mouse strains used are as follows: A, BALB/c; B, C57BL/6; C, C3H; D, PL; E, SJL; F, C57L; G, C57BR; H, SWR; I, *Mus musculus domesticus* (Watkins Star, WSB); J, *Mus musculus brevirostris*; K, SLJ/JLwPt; and L, MA/MyJ. Positions of marker fragments from *Hin*dIII-digested λ phage DNA are indicated on the left (kb, kilobases).

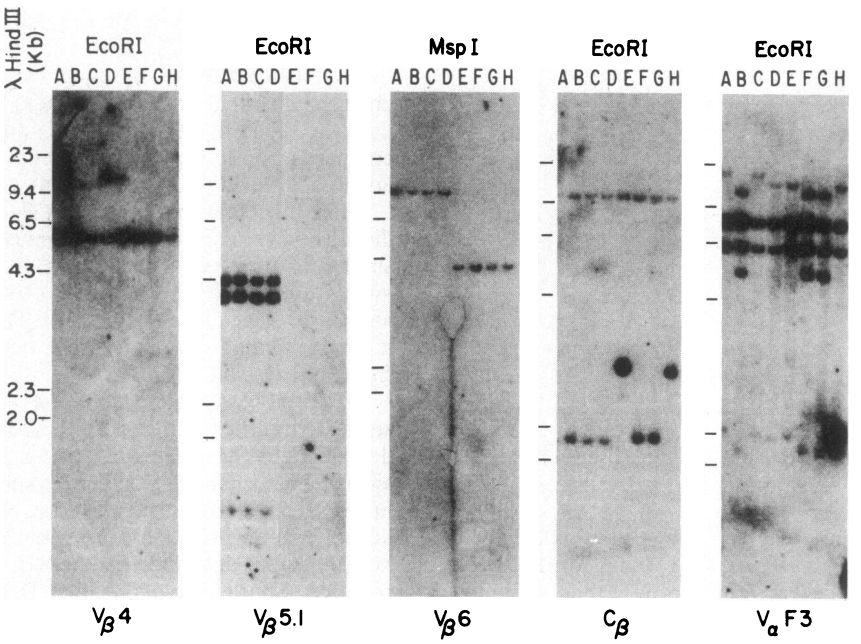

FIG. 3. Genomic Southern blots (18) of liver DNA from eight strains of mice. DNAs were digested with the indicated restriction endonucleases and hybridized to the indicated nick-translated probes. Mouse strains used are as follows: A, BALB/c; B, C57BL/6; C, C3H; D, PL; E, SJL; F, C57L; G, C57BR, and H, SWR.

in BALB/c and SJL DNA are identical when a C_α probe is used (data not shown). Therefore the only common feature of the TCR shared among C57L, C57BR, SJL, and SWR mice that correlates with the lack of reactivity with the KJ16-133 reagent is the set of V_β polymorphisms described here.

DISCUSSION

Murine V_β Gene Family. Thus far, Southern blot analysis has defined two different genotypes at the murine V_β loci. One genotype, present in BALB/c, C57BL/6, C3H, and PL mice, has 20 genes identifiable with 16 different V_β probes. The second genotype, present in SJL, SWR, C57L, and C57BR mice, has only 10 genes identifiable with the same 16 V_β probes. Therefore, 10 genes have been identified in BALB/c that are absent from SJL; we examined 42 V_β–C_β-containing cDNA clones of Con A-stimulated SJL spleen blasts to see if any V_βs could be identified in SJL that were absent from BALB/c. These clones were found to represent repeat usage of 10 different V_β genes, all of which are present in the BALB/c genome. We therefore conclude that the SJL-type genome represents a V_β deletion mutant of the more common genotype represented by BALB/c.

The V_β genes that are shared among SJL, C57BL/6, and BALB/c are highly homologous, with <1% difference found between the V_β7/pHDS11 and V_β3/2B4 alleles of these strains. It therefore seems that, while the SJL mouse has a significant deletion of V_β genes, its existing V_β genes have not diverged to any great extent from strains lacking the deletion event; this suggests either that the deletion event occurred recently or that the murine V_β genes are highly conserved.

In agreement with the observed low level of polymorphism between sequenced V_β genes from different inbred mouse strains, we find very few RFLPs between strains in their V_β loci. Strains known to be different at their *Lyt-2* loci (27) or V_κ loci (28), both linked to the TCR β chain on chromosome 6 (26), are nevertheless identical in their V_β loci to the extent we have examined it. This implies either that the V_β loci are evolving slowly in mice, in contrast with the apparent rapid interspecies divergence reported earlier (7), or that the examined inbred strains share a common ancestry. Interestingly, a limited examination of two wild mice (Fig. 2) failed to reveal any RFLPs distinct from those of the inbred strains. A more thorough examination of wild mice is needed to clarify this point.

Mapping the V_β Deletion. Given the available information from Southern blot analysis, it may be possible to predict the site of the V_β deletion event in SJL. A deletion event occurring adjacent to a V_β gene should change the restriction fragment pattern visualized on a genomic Southern blot using that V_β as a probe. Of the 16 V_β probes available to us, only 4 show RFLPs in SJL with respect to other strains which are not missing V_βs (Table 1). The V_β6/LB2 probe shows a polymorphism with *Msp* I but not *Eco*RI, *Pvu* II, or *Sac* I. This may reflect a simple point mutation or could reflect a more substantial chromosomal rearrangement near this gene. More interestingly, the V_β10 probe shows RFLPs with four enzymes and the V_β1 probe shows RFLPs with three enzymes in SJL DNA with respect to BALB/c. We therefore propose that at least one end of the SJL V_β deletion event occurred near the V_β10 and/or V_β1 genes.

V_β Gene Usage in Spleen. While the relative usage of V_βs isolated from the SJL spleen library varied from 1 to 11 clones out of 42 examined, most V_β genes were present at a level of ≈5–10%. Above this level, V_β6 (LB2) was identified in 11/42 clones (26%) and V_β7 was identified in 7/42 clones (17%). The V_β6 gene also appears to be frequently expressed in other mice, being the most frequently reported V_β gene in the literature, with five independent isolates reported prior to this paper (refs. 5, 7, and 29; S. Hedrick, cited in ref. 7). In contrast, the V_β7 gene has been reported only once by others (20). We note that this gene is represented at <2% among C_β-positive clones in an unamplified library of Con A-stimulated C57BL/6 spleen blasts (data not shown) but that it is present at a level of 17% in a similar SJL library (Table 2). It is not clear if this increase in V_β7 usage is related to the $H\text{-}2^s$ haplotype of the SJL mouse or if it is the result of an antigen-related expansion in compensation for the absence of other V_βs in SJL.

We previously reported a statistical analysis of V_β gene usage, concluding that <30 genes account for most V_β gene expression in the examined inbred mouse strains (5, 6). The accuracy of the statistical analysis depends upon V_βs being equally represented *in vivo*; as seen in Table 2, this is clearly

not the case. Nevertheless, we found that only 10 V_β gene segments accounted for every V_β-containing cDNA clone isolated from an SJL spleen cDNA library. It therefore seems that the prediction of limited V_β gene expression is correct and that this observation becomes even more striking in SJL-type mice, which have deleted ≈50% of their V_β genes.

TCR Idiotype. It has been reported that the rat monoclonal antibody KJ16-133 binds to ≈20% of peripheral T cells in most inbred mice but does not react with any T cells from SJL, SWR, C57L, or C57BR mice (11, 24). It was suggested that this antibody defined a TCR allotype present on a C_β (11) or a J_β (24) element. We note that while two nonconservative changes were seen in SJL J_β and C_β elements when contrasted with C57BL/6, the sequenced genes were nevertheless 99.3% homologous at the amino acid level. Further, it has been shown that RFLPs classify C57BR and C57L as being similar to C57BL/6 and different from SJL at the J_β/C_β locus, even though C57BL/6 reacts with KJ16-133 while the other strains do not. Here we report that SJL, SWR, C57BR, and C57L do share a deletion event involving ≈50% of V_β genes identified to date. We therefore suggest that KJ16-133 binds to one or more V_β segments that are present in BALB/c but absent from SJL.

Since KJ16-133 reacts with ≈20% of peripheral T cells in BALB/c mice, it seems unlikely that it binds a single V_β segment. However, considering the low homology between unrelated V_β genes (20–70%) (6, 7), it also seems unlikely that KJ16-133 reacts with multiple unrelated V_βs. Among the V_βs we report to be missing from the SJL genome are two three-member families ($V_\beta 5$ and $V_\beta 8$), as defined by cross-hybridization on genomic Southern blots. We propose that KJ16-133 reacts with the members of one of these families, and that these three V_β genes together are expressed on 20% of peripheral T cells. Reactivity patterns of functional T cells whose V_β genes have been cloned with KJ16-133 could define which V_β determinants react with this antibody. Of the V_β genes known to be absent from SJL, two have been identified in clonal cell lines. The CTL F3 expresses $V_\beta 11$ (5) but does not react with KJ16-133 (data not shown), whereas the T_H cell line C5, expressing $V_\beta 8.1$ (7), does bind KJ16-133 (G. Freeman, personal communication). We therefore suggest that KJ16-133 may react with a determinant encoded by the three members of the C5/$V_\beta 8$ family. As such, KJ16-133 would be better classified as an anti-idiotypic reagent, as it defines a polyclonal V_β-encoded determinant and does not distinguish an allelic variant.

Immunologic Implications. The SJL-type mice offer a unique system for studying the functional role of V_β genes. It has been shown that a small number of V_β genes account for the bulk of TCR repertoire in inbred mice (5, 6). SJL, SWR, C57L, and C57BR mice are missing 50% of these otherwise highly expressed genes, yet they do not appear to be grossly immunoincompetent animals. This suggests either that the TCR β chain plays a relatively minor role in the functioning of the immune system or that the plasticity in TCR is great enough to accommodate the loss of 50% of V_β genes. It may be that the SJL-type mice are less immunocompetent than other mice and that this genotype would be selected against in the wild, but their immune system is nevertheless adequate for survival in the laboratory. Mice of this V_β genotype would be ideal animals to look for immune response gene-like effects that map to TCR β-chain defects on chromosome 6.

One can predict at least one unique immune response that should be present in SJL-type mice. These mice should respond to determinants encoded by the V_β gene segments that they are missing. Along this line, Bevan and co-workers (12) have characterized a monoclonal antibody (F23.1) derived from a C57L anti-BALB.B immunization that appears to have identical reactivity to the rat monoclonal KJ16-133 (presumably an anti-V$_\beta$8/C5 family reagent). As such, C57L or C57BR should prove to be a valuable source of other anti-V$_\beta$ reagents and would be ideal animals in which to raise TCR anti-clonotypic antibodies, using cell lines expressing V_βs missing in C57L or C57BR.

Note. After submission of this manuscript, additional data were reported which support our conclusion that the KJ16-133 antibody is specific for the three members of the $V_\beta 8$ gene family (30).

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1. Hedrick, S., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **308**, 153–158.
2. Chien, Y., Gascoigne, N., Kavaler, J., Lee, N. E. & Davis, M. M. (1984) *Nature (London)* **309**, 322–326.
3. Tonegawa, S. (1983) *Nature (London)* **302**, 575–581.
4. Seidman, J., Leder, A., Nau, M., Norman, B. & Leder, P. (1978) *Science* **202**, 11–15.
5. Behlke, M., Spinella, D., Chou, H., Sha, W., Hartl, D. & Loh, D. (1985) *Science* **229**, 566–570.
6. Barth, R., Kim, B., Lan, N., Hunkapiller, T., Sobieck, N., Winoto, A., Gershenfeld, H., Okada, C., Hansburg, O., Weissman, I. & Hood, L. (1985) *Nature (London)* **316**, 517–523.
7. Patten, P., Yokota, T., Rothbard, J., Chien, Y., Drai, K. & Davis, M. M. (1984) *Nature (London)* **312**, 40–46.
8. Loh, D., Bothwell, A., White-Scharf, M., Imanishi-Kari, T. & Baltimore, D. (1983) *Cell* **33**, 85–93.
9. Clarke, S., Claflin, J., Potter, M. & Rudikoff, S. (1983) *J. Exp. Med.* **157**, 98–113.
10. Ben-Neriah, Y., Cohen, J., Rechavi, G., Zakut, R. & Givol, D. (1981) *Eur. J. Immunol.* **11**, 1017–1022.
11. Roehm, N., Herron, L., Cambier, J., DiGuisto, D., Haskins, K., Kappler, J. & Marrack, P. (1984) *Cell* **38**, 577–584.
12. Staerz, U., Rammensee, H., Benedetto, J. & Bevan, M. (1985) *J. Immunol.* **134**, 3994–4000.
13. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
14. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
15. Huynh, T. V., Young, R. A. & Davis, R. W. (1984) in *DNA Cloning: A Practical Approach*, ed. Glover, D. (IRL Press, Oxford), pp. 49–78.
16. Gubler, V. & Hoffman, B. (1983) *Gene* **25**, 263–269.
17. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
18. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–518.
19. Goverman, J., Minard, K., Shastri, N., Hunkapiller, T., Hansburg, D., Sercarz, E. & Hood, L. (1985) *Cell* **40**, 859–867.
20. Tonegawa, S. (1984) *Nature (London)* **309**, 757–762.
21. Rinaldy, A., Wallace, B., Simm, M., Becker, A. & Epplen, J. (1985) *Immunogenetics* **21**, 403–406.
22. Siu, G., Kronenberg, M., Strauss, E., Haars, R., Mak, T. W. & Hood, L. (1984) *Nature (London)* **311**, 344–349.
23. Yoshikai, Y., Anatoniou, D., Clark, S., Yanagi, Y., Sangster, R., Van den Elsen, P., Terhorst, C. & Mak, T. (1984) *Nature (London)* **312**, 521–524.
24. Epstein, R., Roehm, N., Marrack, P., Kappler, J., Davis, M., Hedrick, S. & Cohn, M. (1985) *J. Exp. Med* **161**, 1219–1224.
25. Gascoigne, N. R., Chien, Y., Becker, D. M., Kavaler, J. & Davis, M. M. (1984) *Nature (London)* **310**, 387–391.
26. Caccia, N., Kronenberg, M., Saxe, D., Haars, R., Bruns, G., Goverman, J., Malissen, M., Willard, H., Yoshikai, Y., Simon, M., Hood, L. & Mak, T. W. (1984) *Cell* **37**, 1090–1099.
27. Klein, J. (1975) *Biology of the Mouse Histocompatibility-2 Complex* (Springer, New York).
28. Huppi, K., Jouvin-Marche, E., Scott, C., Potter, M. & Weigert, M. (1985) *Immunogenetics* **21**, 445–457.
29. Rupp, F., Acha-Orbea, H., Hengartner, H., Zinkernagel, R. & Joho, R. (1985) *Nature (London)* **315**, 425–427.
30. Sim, G. & Augustin, A. (1985) *Cell* **42**, 89–92.