RIII S/J (H-2')
An Inbred Mouse Strain with a Massive Deletion of
T Cell Receptor Vβ Genes

By TARIQ M. HAQQI, SUBHASHIS BANERJEE, GARY D. ANDERSON,
AND CHELLA S. DAVID

From the Department of Immunology, Mayo Clinic, Rochester, Minnesota 55905

Receptors on a majority of T cells are made up of two polypeptides, α and β, disulfide linked to each other and associated on the plasma membrane with a collection of invariant proteins called CD3 (1). The α and β chains of the TCR are composed of an external NH2-terminal, variable region (V) and an internal COOH-terminal constant (C) region (2). The ligand for the TCR is defined by the combination of antigen and one of the allelic forms of the cell surface products of the MHC (1). The V region of the β chain is encoded by three separate DNA segments, Vβ, diversity (Dβ), and joining (Jβ) that recombine somatically to form the antigen binding domain. Genomic cloning studies of the TCR β chain gene in inbred strains of mice have identified two constant regions termed Cβ1 and Cβ2 (3,4). Clusters of diversity and joining region genes lie several kilobases upstream of each of the constant region genes. In addition, 21 Vβ genes have been identified in most inbred strains of mice (5,6).

Previously, four inbred strains of mice (C57L, C57Br, SWR, and SJL) have been identified in which there is a deletion of ~50% of TCR Vβ genes, including Vβ5 and Vβ8 subfamilies (7). These are also known as KJ16- strains because of lack of reactivity with KJ-16-133 mAb (1). Recently we have added another inbred strain of mouse, AU SS/J (H-2q) to this group (8). Interestingly, all these KJ16-ve strains had the functional allele of Vβ17 gene, while the KJ16+ve strains had the nonfunctional Vβ17b allele (9). Another study showed that natural populations of mice also carry a reduced TCR Vβ gene repertoire, but no new TCR Vβ deletion was found among the inbred strains of mice analyzed in this and a subsequent study using a panel of inbred strains (10,11). We report here the identification of an inbred strain of mouse, RIII S/J (H-2'), with deletion in the TCR Vβ loci encompassing more Vβ genes, than in the previously known TCR Vβ deletion mutants.

Materials and Methods

Mice. RIII S/J and SJL/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in our colony. B10 and B10.RIII mice are bred and maintained in our mouse colony at the Mayo Clinic, Rochester, MN.

This work was supported by National Institutes of Health grants AI-14764 and CA-24473, and by the Minnesota Chapter, Arthritis Foundation and Mayo Foundation. S. Banerjee's present address is Joint Diseases Laboratory, Shriners Hospital for Crippled Children, Montreal, Canada H3G1A6.
Monoclonal Antibodies. F23.1 hybridoma was a kind gift from Dr. J. Bluestone (National Institutes of Health, Bethesda, MD) and detects Vα8 TCR (Vα8.1, 8.2, 8.3). The antibodies were purified from culture supernatants over a protein A-Sepharose column (Pharmacia Fine Chemicals, Piscataway, NJ). KJ23a hybridoma which detects Vβ17a TCR and KJ25 hybridoma which detects Vγ3 were kindly provided by Dr. Philippa Marrack (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). mAb 44-22-1 (12), which detects Vγ6 TCR was a kind gift from Dr. Hans Hengartner (Zurich, Switzerland) and the antibody was used as a culture supernatant. RR-3-15 mAb, which detects Vγ11 TCR was a kind gift of Drs. Kanagawa and Palmer (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). The mAbs were biotinylated as described earlier (13).

Flow Cytometry. Fluorescent staining and flow cytometry were performed as described earlier (13) using FACS-IV flow cytometer (Becton Dickinson & Co., Mountain View, CA) and dual fluorescent contours printed out after gating on lymphocytes by light scatter analysis.

Probes and Purification of Inserts Used as Probes on Southern Blots. Vβ nomenclature is according to Barth et al. (5). The probes, except Vα17a probe, were from a cDNA library subcloned into PUC12 vector and were kindly provided by Dr. Dennis Y. Loh (Howard Hughes Medical Institute, St. Louis, MO). The Cβ1 probe was a 450-bp Eco RI fragment; Vγ6 probe was a 200-bp Hind III fragment; Vγ9 probe was a 380-bp Eco RI-Sac I fragment; the Vβ10 probe was a 269-bp Pst I-Sml I fragment; Vγ13 probe was a 317-bp Rsa I fragment containing 285-bp Vγ13 sequences; Vγ3 probe was a 220-bp Eco RI-MspI fragment and Vγ7 was a 200-bp fragment. Vγ17a probe was a kind gift of Dr. Philippa Marrack (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) and was used after nick translating the whole plasmid. All the inserts were isolated from LMP agarose (Bethesda Research Laboratories [BRL], Gaithersburg, MD) and purified over NAGS columns (BRL) according to suppliers instructions, and were dissolved at a final concentration of 0.1 μg/μl in TE, pH 7.5 (10 mM Tris, pH 7.5; 1 mM EDTA).

Southern Blotting. High molecular weight genomic DNA (>100 kb average) was prepared from the livers of mice by the method of Murray and Kaiser (14). DNAs (10 μg) were digested to completion with Eco RI, Msp I, Hind III, and Kpn I restriction endonucleases (Genomic Grade; International Biotechnologies, Inc., New Haven, CT), separated on 0.8% agarose gels and transferred to nylon membranes (Oncor, Gaithersburg, MD) according to the method of Reed and Mann (15). Blots were probed with random primed (16) probes specific for Cβ and various Vγ genes at 65°C for >12 h (6x SSC, 5x Denhardt's, 1% SDS, 100 μg/ml sheared salmon sperm DNA, 10 mM EDTA). Blots were washed down to 1x SSC at 65°C and exposed overnight using Fuji Rx-G film and Lightning Plus (New England Nuclear, Boston, MA) intensifying screens.

Results

Expression of Vγ TCR on Peripheral Blood Lymphocytes. Dual fluorescence flow cytometry on PBL from B10, B10.RIII, and RIII SJ mice were performed with mAbs detecting Vγ8, Vβ17a, Vγ6, Vγ11, and Vγ3 TCR. F23.1 mAb, detecting Vγ8.1, 8.2, and 8.3, stained 21% of T cells in the PBL of B10 mice but did not stain any of the cells from RIII SJ mice indicating deletion of TCR Vγ8 subfamily in this strain similar to SWR (Fig. 1 a). mAb KJ23a is specific for Vβ17a TCR (9) and Vγ17a allele is not present in B10 and B10.RIII mice, which have the nonfunctional Vγ17b allele. Previously identified TCR deletion mutants SJL, SWR, and C57L express Vγ17a, while in C57Br it is clonally deleted in the context of I-E. The antibody stained 0.0% of RIII, 0.0% of B10 and 14.5% of SWR T cells (Fig. 1 b). This result could have been either due to the clonal elimination of Vγ17a +ve cells in the context of I-E or RIII SJ could have the nonfunctional Vγ17 allele like B10. On the other hand, it could also be due to genomic deletion of Vγ17. Fig. 1 c shows the results of dual fluorescence flow cytometry using mAb 44-22-1, which is specific for Vγ6 TCR. This antibody stained 8.1% of B10 and 0% of RIII and CBA T cells.
Figure 1. Dual fluorescence contours of peripheral blood lymphocytes from B10, SWR, RIII S/J, and CBA mice using (a) anti-Thy 1 (green) and anti-Vβ8-F23.1 (red) antibodies; (b) anti-Thy 1 (green) and anti-Vβ17-KJ-23a (red) antibodies, and (c) anti-Thy 1 (red) and anti-Vγ6-44-22-1 (green) antibodies. Percentages of T cells with F23.1 phenotype (a) are 21 for B10, 0 for SWR, and 0 for RIII S/J. Percentages of T cells with KJ23a phenotype (b) are 0 for B10, 14.5 for SWR, and 0 for RIII S/J. Percentages of T cells with 44-22-1 phenotype (c) are 8.1 for B10, 0 for CBA, and 0 for RIII S/J.

If this was due to clonal deletion of Vβ6+ T cells in the context of Mlsa and I-E similar to CBA, (B10 × RIII S/J)F1 mice should also be negative. Instead, (B10 × RIII S/J)F1 mice showed an intermediate expression of Vβ6+ T cells indicating that they were heterozygous for Vβ6 (not shown). This suggested that in RIII S/J strain, Vβ6 is either nonfunctional (pseudogene) or deleted from the genome. The deletion of Vβ11 gene was shown by using RR-3-15 mAb on PBL from RIII S/J. Finally, an antibody against Vγ3 (KJ 25a) stained Vγ3+ T cells in RIII S/J marking the downstream boundary of the deletion.
Screening of TCR Vβ Genes in RIII S/J DNA. Results of Southern blotting and probing with specific constant region and variable region genes probes are shown in Fig. 2 (a–h). As can be seen from Fig. 2 a, the Cβ probe detected two bands of approximately 9 kb (Cβ2) and 2.2 kb (Cβ1) in B10 and B10.RIII, while in RIII S/J mice the Cβ1 band was ~3.0 kb, suggesting that this region of chromosome 6 (which carries Vβ locus in mice) in RIII S/J is similar to other Vβ TCR deletion mutant strains of mice (11).

Using probes specific for Vβ9 and Vβ13 (Figs. 2, b and c), which are upstream of Vβ6 gene, no signal was detected in the RIII S/J while B10 and B10.RIII gave bands of ~3.0 and 6.0 kb. Thus, RIII S/J lacks Vβ9 and Vβ13 genes like other TCR Vβ deletion mutants. The weakly hybridizing bands in both figures are due to incomplete stripping of another probe. Probes specific for Vβ5 and Vβ8 subfamilies also failed to hybridize in the RIII S/J lane (data not shown) thus confirming the deletion of Vβ8 subfamily as indicated by the absence of F23.1⁺T cells in PBL from RIII S/J (Fig. 1 a). Using probe specific for Vβ10 gene, which is present upstream

![Figure 2](image-url)
of Vβ5 subfamily and is polymorphic between strains with wild-type TCR and TCR deletion mutant strains of mice, single bands were detected on Eco RI–digested genomic DNA in all the lanes with band in RIII S/J showing restriction fragment length polymorphism with respect to B10 and B10.RIII (data not shown).

DNAs digested with Msp I and Eco R1 and probed with Vβ6-specific probe (Fig. 2, d and e) showed that RIII S/J lacks the Vβ6 gene as judged by the absence of hybridizing bands in RIII S/J lane. The Vβ6 band in SJL is in agreement with the earlier reported results (7) where the same restriction fragment length polymorphism (RFLP) was found in the Vβ6 region between KJ-16-ve and KJ-16+ve strains using Msp I enzyme. Thus, in RIII S/J the deletion extends further downstream beyond the Vβ6 gene compared with the other known TCR Vβ deletion mutants. The downstream order of Vβ gene arrangement from Vβ6 gene is Vβ15, Vβ17, Vβ3, and Vβ7 (17). Vβ15-specific probe failed to give a signal in RIII S/J lane (data not shown), while hybridizing to ~6-kb bands in B10 and B10.RIII DNAs digested with Eco RI, indicating that Vβ15 gene is also missing. The Vβ17.1 gene probe used on DNAs digested with Hind III, which detects an RFLP between Vβ17a and Vβ17b alleles (9), shows that Vβ17 gene is also missing in RIII S/J (Fig. 2 f). Probes specific for Vβ3 and Vβ7 genes detected a single hybridizing band in all the lanes (Fig. 2, g and h) marking the 3′ boundary of the deletion in the Vβ locus in RIII S/J mice. Thus, the deletion in RIII S/J mice includes Vβ6, Vβ15, and Vβ17 genes in the downstream direction. The results of this mapping also indicate that RIII S/J mice have lost ~130 kb of the TCR Vβ locus and with it 13 of the 21 known TCR Vβ genes.

Discussion

Earlier studies (7) suggested that there are two different genotypes at Vβ locus of the TCR genes in inbred strains of mice. One genotype represents strains like BALB/c, C57BL/6, C3H, PL, and C57BL/10 mice where there is no deletion of the known TCR Vβ genes (wild-type TCR, KJ-16+ve). The second genotype is represented by mouse strains SJL, SWR/J, C57Br, C57L, and Au/SSJ where there is a deletion of ~50% of known TCR Vβ genes (mutant TCR, KJ-16-ve). These genotypes have recently been referred to as Vβ (wild-type TCR) and Vβ (mutant TCR) (18). We have found a third genotype in RIII S/J strain with a larger deletion, which includes loss of Vβ6, Vβ15, and Vβ17 genes in addition to Vβ genes already deleted in the previously reported TCR deletion mutants. RIII S/J strain thus could be classified as Vβ in accordance with the recently introduced nomenclature.

RIII S/J mice have not been used extensively in immune response studies. Thus, we do not know how this massive deletion of TCR Vβ locus affects their immune status although they seem to be immunocompetent in the laboratory environment. RIII S/J mice have been reported to be resistant to porcine collagen–induced arthritis (CIA) while B10.RIII is susceptible (19). This could be similar to our recent studies where SWR (KJ16 –ve, H-2b) was found to be resistant to CIA while B10.Q was susceptible (13). By analysis of F1, F2, and backcross mice involving SWR and KJ16 +ve strains we have shown a high correlation between the TCR deletion and CIA resistance. We have recently identified another strain, AU SS/J (H-2b), which is also KJ 16 –ve and resistant to CIA (8). RIII S/J has also been shown to be a nonresponder to M. arthritidis mitogen (MAM) while B10.RIII is a responder (20). The TCR involved in the recognition of MAM may map in this deletion. Thus,
RIII S/J mice could be very useful in identifying TCRs involved in various immune responses and diseases. RIII S/J mice could also be used in raising anticonnotypic TCR antibodies. We have initiated genetic analysis using RIII S/J to study the genetic fine structure of this chromosome and screen for recombination between different \( V_\beta \) genes.

**Summary**

We have identified an inbred strain of mouse, RIII S/J (H-2\(^{\text{b}}\)), that has the largest known deletion of the TCR \( V_\beta \) genes by screening with mAb and TCR \( V_\beta \) specific probes. Upon screening of PBL with mAb F23.1, which is specific for \( V_\beta 8 \) TCR, RIII S/J was found to be negative. On further screening with mAb KJ 23a, which is specific for \( V_\beta 17a \) TCR, RIII S/J was completely negative. We next tested RIII S/J with mAb 44-22-1, which is specific for \( V_\beta 6 \) TCR, and found it also to be negative. The \((\text{B}10 \times \text{RIII})F_1\) mice showed a 50% expression of \( V_\beta 6 \) gene, indicating a genomic rather than a clonal deletion. mAb KJ25, detecting \( V_\beta 3 \), was positive in RIII S/J, denoting the downstream boundary for the deletion. Southern blot analysis of liver DNA using TCR \( V_\beta \)-specific probes confirmed the deletion of \( V_\beta 8 \) gene subfamily and \( V_\beta 5 \) gene subfamily, along with \( V_\beta 9, V_\beta 11, V_\beta 12, \) and \( V_\beta 13 \) genes similar to the known TCR \( V_\beta \) deletion mutants (SWR, SJL, C57L, and C57Br). In addition, RIII S/J is missing \( V_\beta 6, V_\beta 15, \) and \( V_\beta 17 \) genes. Our mapping of the deletion indicates that RIII S/J has lost \( \sim 130 \) kb of \( V_\beta \) chromosome and with it 13 \( V_\beta \) genes out of the known 21 \( V_\beta \) genes of the TCR. The deletion is marked by the presence of \( V_\beta 10 \) gene upstream and \( V_\beta 5 \) gene downstream.

The authors thank Drs. Marrack, Hengartner, Palmer and Loh for reagents and gene probes. We are indebted to Wendy Jones for expert technical assistance and Mary Brandt for skillful preparation of manuscript.

Received for publication 11 January 1989 and in revised form 28 February 1989.

**References**

1. Marrack, P., and J. Kappler. 1987. The T cell receptor. *Science (Wash. DC)*. 238:1073.
2. Hedrick, S. M., E. A. Nielsen, J. Kavaler, D. I. Cohen, and M. M. Davis. 1984. Sequence relationship between putative T cell receptor polypeptides and immunoglobulins. *Nature (Lond.)*. 308:153.
3. Gascoigne, N. R., J., Y. H. Chien, D. M. Becker, J. Kavaler, and M. M. Davis. 1984. Genomic organization and sequence of T cell receptor \( \beta \) chain constant and joining region genes. *Nature (Lond.)*. 310:387.
4. Malissen, M., K. Minard, S. Mjoånes, M. Kronenberg, F. Goerman, T. Hunkapiller, M. B. Prystowsky, Y. Yoshiokai, F. Fitch, T. W. Mak, and L. E. Hood. 1984. Mouse T cell antigen receptor: Structure and organization of constant and joining gene segments encoding the \( \beta \) polypeptide. *Cell*. 37:1101.
5. Barth, R. K., B. S. Kim, N. C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenfield, C. Okada, D. Hansburg, K. L. Weissman, and L. Hood. 1985. The murine T cell receptor uses a limited repertoire of expressed \( V_\beta \) gene segments. *Nature (Lond.)*. 316:517.
6. Behlke, M. A., D. G. Spinella, H. S. Chou, W. Sha, D. L. Hartl, and D. Y. Loh. 1985. T cell receptor \( \beta \) chain expression: dependence on relatively few variable region genes. *Science (Wash. DC)*. 229:566.
7. Behlke, M. A., H. S. Chou, K. Huppi, and D. Y. Loh. 1986. Murine T cell receptor mutants with deletions of β chain variable region genes. Proc. Natl. Acad. Sci. USA. 83:767.
8. Haqqi, T. M., S. Banerjee, W. L. Jones, G. Anderson, M. A. Behlke, D. Y. Loh, H. S. Luthra, and C. S. David. 1989. Identification of a T cell receptor deletion mutant, AU/ssJ (H-2a), which is resistant to collagen induced arthritis. Immunogenetics. 29:180.
9. Kappler, J. W., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987. A T cell receptor Vβ segment that imparts reactivity to a class II major histocompatibility complex product. Cell. 49:273.
10. Huppi, K. E., L. A. D’Hoostelaere, B. A. Mock, E. J. Marche, M. A. Behlke, H. S. Chou, R. J. Berry, and D. Y. Loh. 1988. T cell receptor Vβ genes in natural populations of mice. Immunogenetics. 27:51.
11. Singer, P. A., R. J. McEvilly, R. S. Balderas, F. J. Dixon, and A. N. Theofilopolous. 1988. T cell receptor α chain variable region haplotypes of normal and autoimmune laboratory mouse strains. Proc. Natl. Acad. Sci. USA. 85:7729.
12. McDonald, H. R., R. Schneider, R. K. Lees, R. K. Howe, H. A. Orbea, H. Festenstein, R. M. Zinkernagel, and H. Hengartner. 1988. T cell receptor Vβ use predicts reactivity and tolerance to Mls-encoded antigens. Nature (Lond.). 332:40.
13. Banerjee, S., T. M. Haqqi, H. S. Luthra, J. M. Stuart, and C. S. David. 1988. Possible role of Vβ T cell receptor genes in susceptibility to collagen induced arthritis in mice. J. Exp. Med. 167:832.
14. Murray, N. E., and K. Kaiser. 1985. The use of phage lambda replacement vectors in the construction of representative genomic DNA libraries. In DNA Cloning, a Practical Approach. Vol. I. D. M. Glover, editor. IRL Press, Oxford. 1–47.
15. Reed, K. C., and D. A. Mann. 1985. Rapid transfer of DNA from agarose gel to nylon membrane. Nucleic Acids Res. 13:7201.
16. Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 137:265.
17. Chou, H. S., C. A. Nelson, S. A. Godambe, D. D. Chaplin, and D. Y. Loh. 1987. Germ-line organization of the murine T cell receptor β chain genes. Science (Wash. DC). 238:545.
18. Pullen, A. M., P. Marrack, and J. W. Kappler. 1988. The T cell repertoire is heavily influenced by tolerance to polymorphic self antigens. Nature (Lond.). 335:796.
19. Wooley, P. H., H. S. Luthra, M. M. Griffiths, J. M. Stuart, A. Huse, and C. S. David. 1985. Type II collagen induced arthritis in mice. IV. Variations in immunogenetic regulation provide evidence for multiple arthritisogenic epitopes on the collagen molecule. J. Immunol. 135:2443.
20. Cole, B. C., D. R. Kartchner, and D. J. Wells. 1989. Stimulation of mouse lymphocytes by a mitogen derived from mycoplasma arthritis. VII. Responsiveness is associated with expression of products of Vβ gene family present on the α/β T cell receptor (TCR) for an antigen. J. Immunol. In press.