RIII S/J (H-2^r)
An Inbred Mouse Strain with a Massive Deletion of T Cell Receptor Vβ Genes

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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 NH_2-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 KJ-16$^-$ 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-2^q) 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^r), 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.

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Monoclonal Antibodies. F23.1 hybridoma was a kind gift from Dr. J. Bluestone (National Institutes of Health, Bethesda, MD) and detects Vβ8 TCR (Vβ8.1, 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 Vβ3 were kindly provided by Dr. Philippa Marrack (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). mAb 44-22-1 (12), which detects Vβ6 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 Vβ11 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 nomenculature is according to Barth et al. (5). The probes, except Vβ17a 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; Vβ6 probe was a 200-bp Hind III fragment; Vβ9 probe was a 380-bp Eco RI-Sac I fragment; the Vβ10 probe was a 269-bp Pst I-Smn I fragment; Vβ13 probe was a 317-bp Rsa I fragment containing 285-bp Vβ13 sequences; Vβ3 probe was a 220-bp Eco RI-MspI fragment and Vβ7 was a 200-bp fragment. Vβ17a 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 NACS 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 Vβ genes at 65°C for >12 h (6× SSC, 5× Denhardt's, 1% SDS, 100 μg/ml sheared salmon sperm DNA, 10 mM EDTA). Blots were washed down to 1× 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 Vβ TCR on Peripheral Blood Lymphocytes. Dual fluorescence flow cytometry on PBL from B10, B10.RIII, and RIII S/J mice were performed with mAbs detecting Vβ8, Vβ17a, Vβ6, Vβ11, and Vβ3 TCR. F23.1 mAb, detecting Vβ8.1, 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 S/J mice indicating deletion of TCR Vβ8 subfamily in this strain similar to SWR (Fig. 1 *a*). mAb KJ23a is specific for Vβ17a TCR (9) and Vβ17a allele is not present in B10 and B10.RIII mice, which have the nonfunctional Vβ17b allele. Previously identified TCR deletion mutants SJL, SWR, and C57L express Vβ17a, 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 Vβ17a +ve cells in the context of I-E or RIII S/J could have the nonfunctional Vβ17 allele like B10. On the other hand, it could also be due to genomic deletion of Vβ17. Fig. 1 *c* shows the results of dual fluorescence flow cytometry using mAb 44-22-1, which is specific for Vβ6 TCR. This antibody stained 8.1% of B10 and 0% of RIII and CBA T cells.

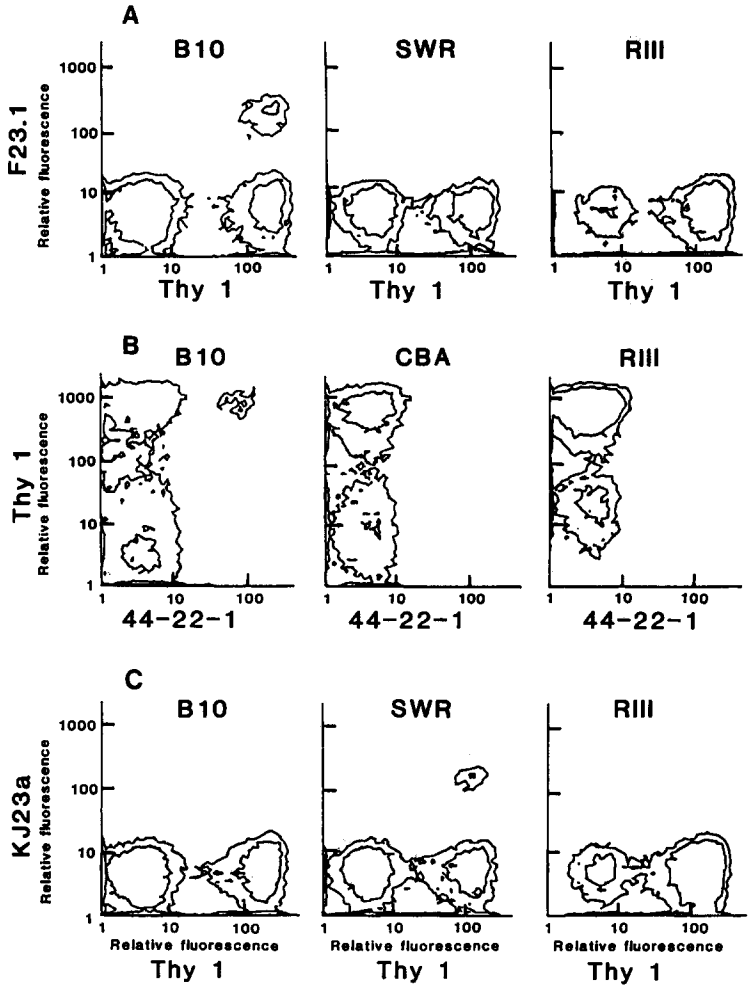

FIGURE 1. Dual fluorescence contours of peripheral blood lymphocytes from B10, SWR, RIII S/J, and CBA mice using (*a*) anti-Thy.1 (green) and anti-Vβ8-F23.1 (red) antibodies; (*b*) anti-Thy.1 (green) and anti-Vβ17-KJ-23a (red) antibodies, and (*c*) anti-Thy.1 (red) and anti-Vβ6-44-22-1 (green) antibodies. 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 Mls^a and I-E similar to CBA, (B10 × RIII S/J)F$_1$ mice should also be negative. Instead, (B10 × RIII S/J)F$_1$ 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 RIIIS/J. Finally, an antibody against Vβ3 (KJ 25a) stained Vβ3$^+$ 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

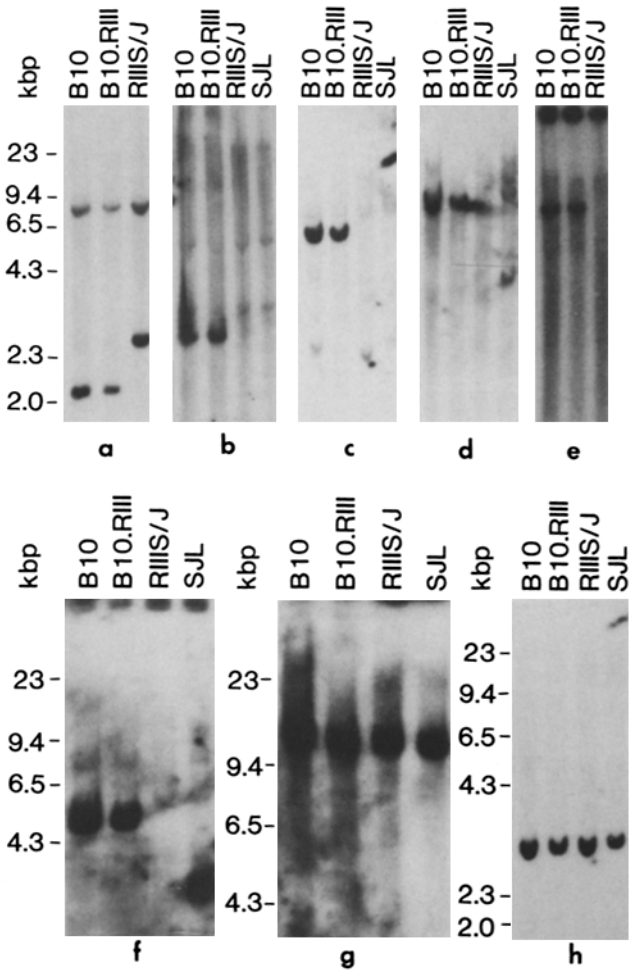

FIGURE 2. Results of genomic Southern blot analysis of liver DNA from B10, B10.RIII, RIII S/J, and SJL/J strains of mice. DNAs were digested to completion with Eco RI (*a–c*, *e*, *h*), Msp I (*d*), Hind III (*f*), and Kpn I (*g*) and the blots were probed with (*a*) Cβ1 probe; (*b*) Vβ9 probe; (*c*) Vβ13 probe; (*d* and *e*) Vβ6 probe; (*f*) Vβ17 probe; (*g*) Vβ3 probe, and (*h*) Vβ7 probe.

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 RI 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 V_β^b (wild-type TCR) and V_β^a (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 V_β^c 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)[1] while B10.RIII is susceptible (19). This could be similar to our recent studies where SWR (KJ16 −ve, H-2^q) was found to be resistant to CIA while B10.Q was susceptible (13). By analysis of F_1, F_2, 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-2^q) 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 anticlonotypic 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 Vβ genes.

Summary

We have identified an inbred strain of mouse, RIII S/J (H-2^r), that has the largest known deletion of the TCR Vβ genes by screening with mAb and TCR Vβ specific probes. Upon screening of PBL with mAb F23.1, which is specific for Vβ8 TCR, RIII S/J was found to be negative. On further screening with mAb KJ 23a, which is specific for Vβ17a TCR, RIII S/J was completely negative. We next tested RIII S/J with mAb 44-22-1, which is specific for Vβ6 TCR, and found it also to be negative. The (B10 × RIII)F$_1$ mice showed a 50% expression of Vβ6 gene, indicating a genomic rather than a clonal deletion. mAb KJ25, detecting Vβ3, was positive in RIII S/J, denoting the downstream boundary for the deletion. Southern blot analysis of liver DNA using TCR Vβ-specific probes confirmed the deletion of Vβ8 gene subfamily and Vβ5 gene subfamily, along with Vβ9, Vβ11, Vβ12, and Vβ13 genes similar to the known TCR Vβ deletion mutants (SWR, SJL, C57L, and C57Br). In addition, RIII S/J is missing Vβ6, Vβ15, and Vβ17 genes. Our mapping of the deletion indicates that RIII S/J has lost ~130 kb of Vβ chromosome and with it 13 Vβ genes out of the known 21 Vβ genes of the TCR. The deletion is marked by the presence of Vβ10 gene upstream and Vβ3 gene downstream.

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