

## DELINEATION OF A DEFECT IN T CELL RECEPTOR $\beta$ GENES OF NZW MICE PREDISPOSED TO AUTOIMMUNITY

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Inbred mice that spontaneously develop a disease similar to SLE have been an important model for elucidating the pathogenesis of this disorder and for the development and testing of various immunologic concepts. The basic abnormality in these mice is B cell hyperactivity leading to autoantibody production and death, primarily via immune complex disease. Despite intensive efforts over the years and descriptions of various B and T cell abnormalities, the molecular basis of this disease remains unknown (reviewed in 1). In an attempt to define their molecular defects, we recently began an analysis of the antigen receptor genes of B and T cells from these mice. Our initial efforts focused on Ig gene structure and regulation, including  $V_H$ - $V_L$  gene structure and repertoire. The results showed that essentially all established Igh-V gene families are identical in lupus and haplotype-matched ancestral normal mice (2), and that the autoantibodies are multigenic (3).

Since T cells are essential for the development of murine SLE (4-7), we are also analyzing autoimmune T cell populations for possible defects, specifically at genetic loci coding for the T cell antigen receptor, whose molecular structure has recently been identified. Three related gene families are expressed in T cells; two encoding the T cell receptor  $\alpha$  and  $\beta$  chains (8, 9), and a third,  $\gamma$  (10), whose function is unknown. Each of these polypeptide chains can be divided into V and C regions. The V regions are encoded by  $V\beta$ ,  $D\beta$  and  $J\beta$  gene segments that rearrange during T cell differentiation to form a functional  $V\beta$  gene (11, 12). There are two closely linked  $C\beta$  genes,  $C\beta 1$  and  $C\beta 2$ , each associated with a cluster of six functional  $J\beta$  gene segments. Each  $J\beta$  cluster has a single  $D\beta$  gene segment located ~500-700 nucleotides upstream (13). Similarly, the  $\alpha$  chain seems to consist of V, D, J, and C regions, with the numbers of J and the V segments apparently larger than those of the  $\beta$  chains (14-16). Complete  $\alpha$  chains can be assembled with or without the participation of D segments, and it appears that there is only one  $C\alpha$  gene (15). In this report, we use restriction fragment length polymorphism (RFLP)<sup>1</sup> analysis to describe the structural continuity of

This is publication 4122 from the Department of Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037. The work reported herein was supported, in part, by grants AM31203, AM33826, and AI07007 from the National Institutes of Health.

<sup>1</sup> Abbreviation used in this paper: RFLP, restriction fragment length polymorphism.

the  $\alpha$  and  $\beta$  T cell receptor locus in autoimmune and ancestral normal strains of mice. A deletion in the NZW T cell receptor  $\beta$  gene locus thus identified was shown by nucleotide sequence analysis to encompass C $\beta$ 1, D $\beta$ 2 and J $\beta$ 2 elements.

### Materials and Methods

**Mouse Strains and Restriction Enzyme Analyses.** Mice used in this study were obtained from the Jackson Laboratory, Bar Harbor, ME, and maintained at the Scripps Clinic and Research Foundation Mouse Breeding Colony. The generation of the BXSB, MRL (MRL/n and MRL-*lpr* lines), NZB and (NZB  $\times$  W) $F_1$  lupus mice and *lpr* congenic strains of normal mice has been described (17–19). All RFLP analyses were performed on germline liver DNA. High molecular weight liver DNA was prepared, digested with restriction enzymes, separated on agarose gels, transferred to nitrocellulose filters and probed for T cell receptor sequences essentially as previously described (2). Probes were prepared from cDNA clones derived from a BALB/c thymocyte library. The  $\beta$  probe consisted of a D $\beta$ 2-J $\beta$ 2-C $\beta$ 2-encoding fragment. The  $\alpha$  probe consisted of a fragment extending through the C region and including the first 10 nucleotides of a putative J region (our unpublished data).

**Subcloning and Sequencing.** Liver DNA from NZW mice was double-digested with the restriction enzymes Hind III and Eco RI, separated on a 0.7% agarose gel, and DNA corresponding to the 3 kb C $\beta$  hybridizing fragment was eluted using the DE81 paper technique (20). Isolated DNA was subcloned into the Hind III/Eco RI sites of a pUC 12 vector (21), and after transformation into a bacterial host, clones containing  $\beta$  chain T cell receptor sequences were identified by colony screening (22) using the  $\beta$  chain cDNA probe. Plasmid DNA from positive clones was isolated (23), digested with the appropriate restriction enzymes, terminally labeled with  $^{32}$ P using either polynucleotide kinase or DNA polymerase I Klenow fragment, and sequenced by the partial chemical degradation method of Maxam and Gilbert (24).

### Results

In an initial structural analysis of the T cell receptor locus, we screened 18 strains of mice for gross alterations in T cell receptor  $\alpha$  and  $\beta$  genes. Included were the classical autoimmune strains [NZB, (NZB  $\times$  W) $F_1$ , BXSB, MRL-*lpr/lpr*, MRL-+/+], autoimmune mice of normal background homozygous for the *lpr* gene (C57BL/6-*lpr/lpr*, AKR-*lpr/lpr*, C3H/HeJ-*lpr/lpr*), and ancestral normal strains. Hybridization of the  $\alpha$  chain probe to Eco RI (Fig. 1A) Bam HI- or Hind III- (not shown) digested DNA identified genomic DNA fragments encoding the C $\alpha$  gene but revealed no polymorphisms among the strains tested. Hybridization of genomic DNA with a T cell receptor  $\beta$  chain probe, capable of detecting C $\beta$ 1, C $\beta$ 2, D $\beta$ 2, and J $\beta$ 2 genes, to genomic DNAs digested with Bam HI (Fig. 1B) disclosed nonpolymorphic DNA fragments in 16 of the 18 strains tested. The two exceptions were NZW mice, in which only one hybridizable band was detected, and (NZB  $\times$  W) $F_1$  mice, which had the expected composite profile indicating the presence of both parental alleles.

When Eco RI and Hind III digested genomic DNAs were probed, none of the 16 strains showed any polymorphisms (data not shown). However, both digests were useful in further characterizing the anomalous C $\beta$  locus of NZW mice. Fig. 2 is a comparison of restriction enzyme digested DNA from NZB, NZW, and (B  $\times$  W) $F_1$  mice. The hybridizing bands in NZB DNA matched the published (11, 12) restriction map of the C $\beta$  locus (Fig. 2), and were identified as follows: the C $\beta$ 1 gene was on the 2.2, 9, and 10 kb fragments in the Eco RI, Hind III, and

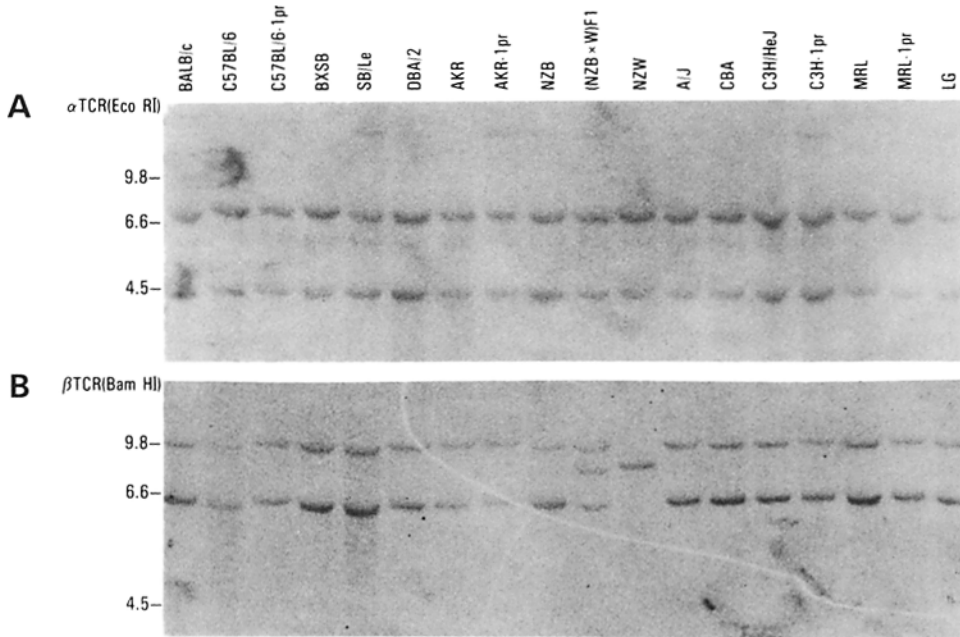


FIGURE 1. RFLP analysis of autoimmune, *lpr* congenic, and normal background mice. Size-separated liver DNA digested with either Eco RI (A) or Bam HI (B) was probed with either an  $\alpha$  chain C region cDNA clone (A) or a  $\beta$  chain cDNA clone containing C $\beta$ 2, J $\beta$ 2, D $\beta$ 2 structures (B). Size markers (in kb) (Hind III-digested  $\lambda$  DNA) are on the left side of each blot.

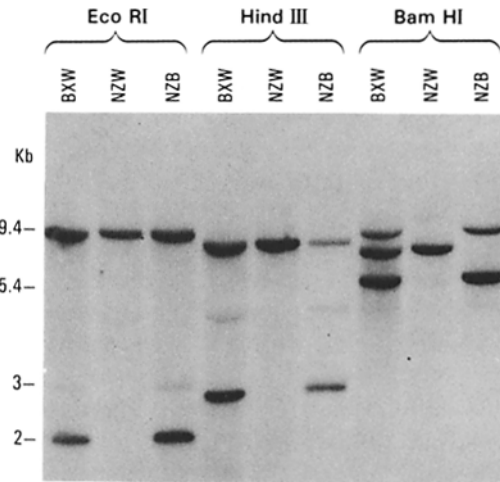


FIGURE 2. RFLP analysis of NZB, NZW, and (NZB × W)<sub>1</sub>F<sub>1</sub> size-separated liver DNA probed with the  $\beta$  chain cDNA probe described in Fig. 1. Size markers (in kb) are given on the left side of the autoradiograph, and below is the restriction map of the  $\beta$  chain locus. Abbreviations: R, Eco RI; B, Bam HI; K, Kpn I; H, Hind III.

Bam HI profiles, respectively; similarly, the  $C\beta 2$  gene was on the 9.4 kb Eco RI, 3 kb Hind III, and 6 kb Bam HI fragments. The weakly hybridizing 3 kb and 5 kb bands in the Eco RI and Hind III profiles contained the  $D\beta 2$  and  $D\beta 2$ - $J\beta 2$  regions, respectively, with which our probe had a short stretch of homology. In the NZB Bam HI profile, the  $D\beta 2$ - $J\beta 2$  region was included in the 10.5 kb fragment together with the  $C\beta 1$  complex. In contrast to the NZB profiles, NZW DNA showed only a single hybridizing band with each of the restriction enzymes used. In the Eco RI and Hind III lanes (Fig. 2), the hybridizing bands in NZW DNA comigrate with the 9.4 and 9 kb bands present in the NZB profiles. However, with reference to a restriction map of the  $C\beta$  locus, it became evident that these bands could not be identical to their counterparts in NZB DNA, but must instead represent composites encompassing portions of the two restriction fragments that normally contain the  $C\beta 1$  and  $C\beta 2$  genes (i.e., the 9.4 and 2.2 kb Eco RI fragments, and the 9 and 3 kb Hind III fragments). The NZW Eco RI and Hind III profiles also showed loss of the weakly hybridizing  $D\beta 2$  region bands. These results, together with the NZW Bam HI profile, indicating probable loss of a site in the  $D\beta 2$  region, were most consistent with the occurrence in the NZW  $C\beta$  locus of a large (~8 kb) deletion, beginning somewhere between the two Eco RI sites near the  $C\beta 1$  gene, and extending downstream to somewhere between the two Hind III sites flanking the  $C\beta 2$  gene. Such a deletion would entirely remove the  $D\beta 2$ - $J\beta 2$  elements from the NZW  $C\beta$  locus. The possibility that the deletion also included the  $D\beta 1$ - $J\beta 1$  region was ruled out, based on our finding that NZW  $\beta$  chain cDNA clones contain normal  $D\beta 1$ - $J\beta 1$  sequence elements (our unpublished observation). In addition, our restriction analysis of such cDNA clones determined that NZW mice produce normal  $\beta$  chain mRNA of the  $C\beta 2$  type (lacking an Eco RI site in the C-terminal coding sequence). It seemed likely, therefore, that the putative deletion occurred between homologous flanking or internal sequence elements of the  $C\beta 1$  and  $C\beta 2$  genes, leaving either an intact  $C\beta 2$  gene or creating a hybrid gene composed of upstream  $C\beta 1$  elements and downstream  $C\beta 2$  elements.

For more precise definition of the deletion in the NZW  $C\beta$  locus, we cloned and sequenced the genomic fragment containing the NZW  $C\beta$  gene. Genomic DNA was digested with Eco RI and Hind III, and the fragments were separated by preparative agarose gel electrophoresis. A 3 kb fragment containing the gene was isolated, subcloned into the pUC12 plasmid vector, and sequenced by the method of Maxam and Gilbert (24). The intron/exon organization of the gene, sequencing strategy, and alignment of the NZW  $C\beta$  nucleotide sequence with the published  $C\beta 1$  and  $C\beta 2$  gene sequences (11, 12) are shown in Fig. 3. The intron/exon structure of the NZW gene was indistinguishable from that for  $C\beta 1$  and  $C\beta 2$ . As shown (Fig. 3), the sequence 5' of exon 1 in the NZW gene was identical to that of  $C\beta 1$ , and not  $C\beta 2$ , whereas the sequence 3' of exon 1 (in the first intron) matched that of  $C\beta 2$ . Two polymorphic residues identified within exon 1 of the  $C\beta$  gene, one at position 261 (C in  $C\beta 1$ , A in  $C\beta 2$ ) and another at position 149 (A in  $C\beta 1$  and in several reported  $C\beta 2$  sequences, G in one  $C\beta 2$  cDNA sequence [10]) could be used to further map the NZW deletion. The NZW  $C\beta$  sequence corresponded to  $C\beta 2$  at both of these positions, thus placing

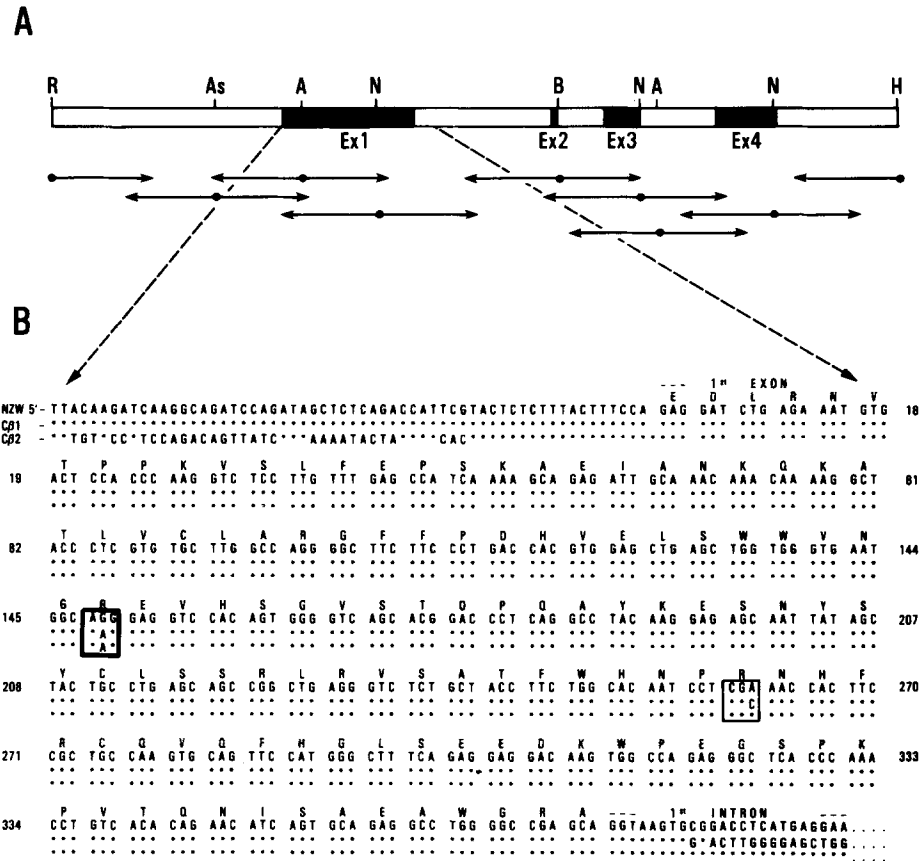


FIGURE 3. Sequencing strategy (A) and comparison of relevant first exon NZW sequence (B) with that published for *Cβ1* and *Cβ2* genes. The intron (open lines)/exon (shaded lines) conformation of the NZW *Cβ* locus, along with specific restriction sites used in sequencing analysis appeared in A. In B is a single letter amino acid code for the first exon sequence above the nucleotide sequence; asterisks below denote sequence homology. Identified polymorphisms in the first exon structure have been boxed. Abbreviations: R, Eco RI; As, Asp 718; A, Ava II; N, Nco I; B, Bgl II; Ex1, Exon 1.

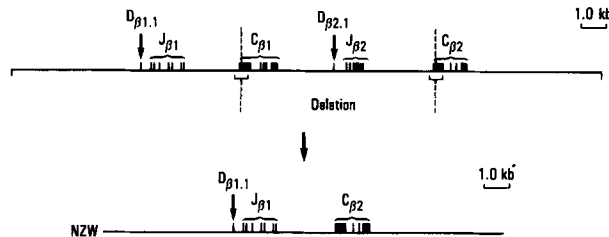


FIGURE 4. A schematic representation of the T cell receptor  $\beta$  chain deletion identified in NZW mice. Hatched lines denote breakpoint regions as determined by previous sequence analysis.

the deleted region between positions -18 and 149 of exon 1, a region of complete homology between  $C\beta 1$  and  $C\beta 2$  (Fig. 4).

### Discussion

Prior immunologic data have clearly shown that murine lupus is T cell-dependent, accompanied in many instances by T cell subset phenotypic and functional abnormalities (reviewed in 1, and 4-7). It is therefore likely that abnormalities in antigen recognition by T cells on the level of T cell receptor genes may contribute significantly to the pathogenesis of SLE. Within the limits of RFLP analysis, the C region loci of the T cell receptor  $\alpha$  and  $\beta$  chains appear highly conserved among autoimmune, *lpr* congenic, and normal strains of mice, with the single exception of the  $\beta$  chain deletion observed in NZW mice. The only other documented restriction polymorphism in a murine T cell receptor C region locus was that of restriction polymorphism at the  $\beta$  chain locus in SJL/J mice (25). This high degree of conservation in the murine T cell receptor C region locus contrasts with that found in humans, where multiple restriction polymorphisms have been identified at both  $\alpha$  and  $\beta$  chain loci (26, 27). Due to the limited sample size thus far studied in both populations, and to the restricted ancestry of laboratory mice, the significance of this observation remains to be determined.

The NZW T cell receptor  $\beta$  chain deletion was further characterized using Southern blotting and DNA sequence analysis. In this strain, an 8 kb deletion has eliminated one of the two  $C\beta$  genes, as well as a complete set ( $D\beta 2$ - $J\beta 2$ ) of D and J region elements. During the completion of this work, Kotzin et al. (28), using Southern blot analysis, also described a deletion of the  $C\beta 1$ - $D\beta 2$ - $J\beta 2$  genes in NZW mice. Our analysis additionally establishes that the NZW  $\beta$  gene deletion originates within the first 167 base pairs of exon 1 of the  $C\beta 1$  gene (residues -18 through 149), and extends to the complementary region within the first exon of the  $C\beta 2$  gene. The precise nucleotide sequence of the gene is maintained without insertions or deletions. A meiotic recombination event can be invoked to explain the deletion with unequal crossing over between the precisely aligned first exons of the  $C\beta 1$  gene on one chromatid and  $C\beta 2$  gene on its homolog, producing a  $C\beta$  locus lacking  $C\beta 1$ ,  $D\beta 2$  and  $J\beta 2$  regions.

Deletion of the  $D\beta 2$ - $J\beta 2$  elements in NZW mice would, in theory, significantly limit T cell receptor  $\beta$  chain diversity. In normal mice (11, 12) and man (29), two  $C\beta$  genes are present with their own single D elements and six functional J elements. As in the case of Ig genes, functional  $\beta$  chain genes are assembled by joining a V region with a D and J element. Diversity is generated first by the choice of V gene, second by the choice of J element, and third by combinatorial and junctional diversity of D-J joining. Recent estimates for the limited number of  $V\beta$  region genes (30, 31) appear to magnify the role of  $D\beta$  and  $J\beta$  elements in the generation of diversity. Analysis of many  $\beta$  chain cDNA clones has indicated that the D elements can randomly combine with any downstream J element (30), totaling 18 possible combinations (six each of  $D\beta 1$ - $J\beta 1$ ,  $D\beta 2$ - $J\beta 2$ , and  $D\beta 1$ - $J\beta 2$ ). Consistent with this is the observation that  $J\beta 2$  elements are twice as frequent in

functional  $\beta$  chain genes as  $J\beta 1$  elements (30). In NZW, only  $D\beta 1$ - $J\beta 1$  joinings are possible, thus these mice may be able to generate only one-third the T cell receptor  $\beta$  chain diversity of other strains. In spite of this seemingly dramatic impairment, the effects on T cell function in NZW mice, if any, are not readily apparent. These mice exhibit near-normal cell-mediated (32) and humoral responses to both thymus-dependent and -independent antigens (33), both quantitatively and qualitatively (IgG subclass distribution). This lack of apparent defect in T cell-mediated immune response in NZW mice suggests that  $V\beta$  genes might adequately generate  $\beta$  chain diversity, and that T cell receptor function, as previously suggested (34, 35), is independent of the  $C\beta$  gene used by the effector cell. Alternatively, sufficient diversity might be contributed by the  $\alpha$  chain V genes and J elements, which appear to be both numerous and polymorphic enough to have this potential (14-16).

The significance of the NZW  $\beta$  chain gene deletion to autoimmunity remains to be determined. Although the longevity of NZW mice is near normal, a significant proportion (~50%) develop, late in life, autoantibodies and a moderate to severe immune complex-type glomerulonephritis. Moreover, NZW mice contribute significantly to the genetic abnormalities associated with early-life severe lupus in the classical SLE hybrid (NZB  $\times$  W) $F_1$ , as well as in hybrids of other SLE-prone strains such as BXSB (1, 36). Furthermore, polyclonal mitogens and certain viruses can induce early autoimmunity and death in NZW (and other lupus background mice), but not in similarly treated normal strains (1, 37, 38). Whether the T cell receptor defect described here is involved in the NZW autoimmune predisposition is not clear. However, the fact that the  $\beta$  gene defect was not found in other lupus strains (BXSB, MRL) does not exclude its possible involvement in the autoimmunity of New Zealand mice. Many observations have indicated that, although the disease in all of these mice may be expressed as a lupus-like syndrome, the abnormalities and pathways of induction in each strain might be different (1).

### Summary

In an attempt to determine whether genes involved in T cell antigen recognition are structurally abnormal and thereby promote murine systemic lupus, we analyzed the structural integrity of the D, J, and C region elements of the T cell receptor  $\alpha$  and  $\beta$  chain genes in all major lupus strains and several normal strains. Within the limits of restriction fragment length polymorphism analysis, all strains had an identical genomic organization, except the NZW mice, in which a deletion of the  $C\beta 1$ - $D\beta 2$ - $J\beta 2$  elements was found. Sequence analysis of NZW genomic elements containing this deletion placed its probable origin within the first exon of  $C\beta 1$ , and extending to a complementary region within the first exon of  $C\beta 2$ . The significance of this abnormality in the pathogenesis of systemic autoimmune disease remains to be determined.

*Received for publication 4 October 1985.*

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