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GENOMIC ORGANIZATION AND EXPRESSION OF ANTIGEN RECEPTOR GENES IN MURINE LUPUS

ARGYRIOS N. THEOFILOPOULOS, REINHARD KOFLER, PAUL A. SINGER, DANIEL J. NOONAN & FRANK J. DIXON

Departament of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037, USA

The extensive analyses of the serologic, cellular, viral and genetic characteristics of lupus mice have provided a very good picture of the immunopathologic characteristics of this disease, but have failed to provide definitive answers with regard to etiology. Nevertheless, the studies performed to date have allowed several conclusions to be drawn which can now form the foundation of current and future etiologic studies. The results from these conventional studies have been reviewed in detail elsewhere (Theofilopoulos & Dixon, 1985), with the main conclusions being as follows: 1) There is a genetic predisposition toward the disease which can be traced to the hematopoeitic stem or lymphoid precursor cells (Eisenberg et al., 1980; Morton & Siegel, 1974; Akizuki et al., 1978; Ikeahara et al., 1985). 2) There is a B cell hyperactivity leading to hypergammaglobulinemia and the production of a variety of autoantibodies (Theofilopoulos et al., 1980). 3) *In vitro* and *in vivo* studies on signal requirement for immune responses (Prud'homme et al., 1983a, b), the role of the thymus in the disease process (Steinberg et al., 1980; Theofilopoulos et al., 1981), and the effects of anti-Thy1 and anti-L3T4 antibodies (Wofsy & Seaman, 1985) have suggested that T cells play a critical role in the murine lupus-associated hypergammaglobulinemia and autoantibody production, and 4) A variety of genetic and nongenetic factors (such as abnormal genes, hormones, bacterial products and viruses) can independently accelerate the autoimmune disease in these mice. The background genome, however, profoundly alters the effects of these accelerators (Hang et al., 1985).

The advent of molecular techniques led us to initiate studies to define these defects at the molecular genetic level. Because of the enormous complexities of these disorders, several avenues have been pursued, but the main emphasis in our laboratory has presently been placed on immunologically related genes and their products, i.e., antigen receptor genes of B and T cells and autoantibody-specific genes.

Immunoglobulin Germline Genes in Autoimmune Mice – The apparently central role of Ig hyperproduction, particularly the high levels of autoantibodies in murine and human lupus, suggest a possible contribution of abnormalities in structural and/or regulatory sequences of Ig genes in the pathogenesis of this disorder. To examine this possibility, high molecular weight DNA was prepared from livers of all major lupus strains, other *lpr* gene homozygous strains which manifest autoimmunity, and their non-autoimmune ancestors, and subjected to restriction fragment length polymorphism (RFLP) analyses (Kofler et al., 1985b) using DNA probes corresponding to 7 variable (V) heavy (H) gene families (36-60, X24, J606, J558, S107, Q 52, 7183) defined by Brodeur & Riblet (1984). Two additional families have recently been described (Winter et al., 1985). Genes within a V_H family share >80% sequence homology, while the sequence homology between families is generally <70%. The complexity of each family differs, wich the J558 family composed of a mininum of 60 and possibly as high as 1,000 nembers (Livant et al., 1986), the J606, Q52 and 7183 approximately 10-15 members each, the 36-60 and S107 having 4-5 members, and the X24 only 2.

Autoradiographs of restriction enzyme-digested size-separated, genomic DNA from lupus mice, their ancestors, and other non-autoimmune strains probed with ^{32}P-labeled cDNA probes corresponding to 7 murine VH gene families are shown in Fig. 1A, B, and C. For brevity, only a representative autoradiograph for each family corresponding to 1 of 3 restriction enzymes (Hind III, EcoRI, BamHI) is depicted. It can be seen that all autoimmune mice revealed restriction fragment patterns identical to those of their non-autoimmune ancestors or, in the case of strains with unknown derivations, closely resembling those of normal

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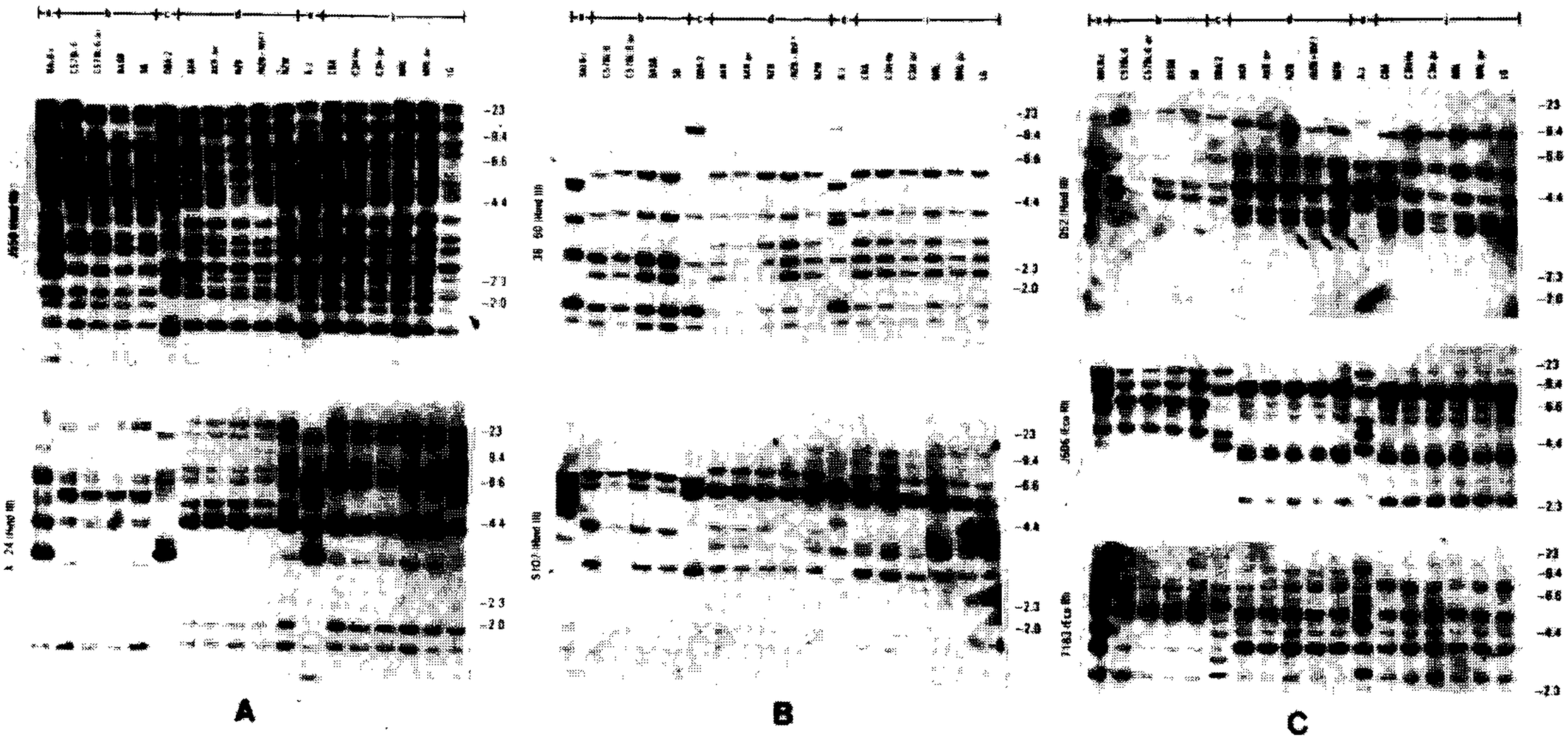

Figs. 1A, B, C: Autoradiographs of restriction enzyme-digested, size-separated liver DNA from several lupus and other inbred strains of mice probed with ^{32}P-labeled DNA corresponding to seven murine V_H gene families. The strains have been organized according to their Ig-V_H haplotypes (indicated by lower case letters between bars above strain designations). The restriction enzyme used for digestion, and the V_H gene family probed are indicated on the left, and size markers on the right side of each blot. Arrows in the autoradiography corresponding to the Q52 family highlight an additional band in all three NZ strains that is not present in the Igh-V^d prototype strain, AKR.

strains. MRL mice, descendend from LG (75%), AKR (13%), C3H (12%) and C57BL/6 (0./3%) progenitors, shared identical patterns with C3H and LG. This places their haplotype as Ighj. BXSB mice completely matched their 2 ancestors, C57BL/6 and SB/Le (Igh^b haplotype). The New Zealand strains (Igh^n or Igh$^{d/e}$ haplotype), with unknown derivation, showed identical restriction fragment patterns with the AKR strain. Finally, introduction of the *lpr* gene, associated with excessive Ig levels and high titers of autoantibodies, did not alter the Ig-V_H restriction fragment patterns compared to the non-*lpr* parental background strains. The only exception was an extra band with NZ genomic DNA and the Q52 probe, which probably reflects the normal rate of divergence of V_H gene families in murine species. Our more recent studies (in preparation) with probes corresponding to the eighth and ninth and a possible tenth V_H gene family (Kofler et al., in preparation) have also failed to demonstrate any differences in Ig gene organization between lupus and normal strains of mice.

Based upon, and hence within the limitations of, RFLP analysis, our and two additional studies (Trepicchio & Barrett, 1985; Painter et al., 1986) suggests that the Ig-V_H gene complex in lupus mice may be essentially normal. This technique can reveal recombinations, gene loss or duplication, sequence alternations and even single point mutations, provided these changes detectably alter the length of restriction fragment hybridizing to the labeled DNA probes. Differences that do not fulfill these requirements will remain undetected, and yet might still contribute to the serologic abnormalities associated with this disease. However, the autoantibody response in murine lupus seems to employ a significant number of V_H genes from various V_H gene families (see below). Possible causal defects (such as lupus-specific V_H genes or sequence alterations in multiple V_H genes) should have become apparent, particularly since three restriction enzymes and ten DNA probes that recognize all known V_H genes within this relatively small chromosomal region were used.

Another possibility for the generation of hypergammaglobulinemia and pathogenic autoantibodies is through germline DNA defects in regulatory sequences controlling Ig gene expression such as promoters, enhancers and switch regions. Murine lupus shows: 1) a good correlation between disease onset and switch of polyclonal and autoantibody Ig from predominantly IgM to predominantly IgG (Steward & Hay, 1976; Papoian, et al., 1977); 2) IgG, but not IgM, levels have an excellent predictive value for disease expression and survival of lupus mice (Theofilopoulos et al., 1985); and 3) most Ig deposits in diseased kidneys are of the IgG isotype, specifically IgG2a-IgG2b (Slack et

al., 1984). These finding suggest that expression of self-directed V_H regions may be pathogenic only in the context of particular C region sequences. Since the enzyme(s) mediating class switch recognize specific DNA sequences, alterations in these sequences could influence this process. However, our comparative RFLP analysis of lupus genomic DNA with appropriate probes to switch regions of the various murine Ig isotypes did not reveal any evidence of gross abnormalities in the respective DNA portions, although an allotypically related polymorphism in $S\mu$ and $S\alpha$ was noted among the various strains tested (data not shown). Therefore, it is more likely that such enhanced switching in lupus occurs under the influence of abnormal levels of, or response to, T cell-derived factors, as we have already suggested (Theofilopoulos et al., 1983 a, b).

The possibility of germline abnormalities in the enhancer elements located within the large joining (J)-constant (C) intron of both H and κ light (L) chain genes was also investigated. Cloning and sequencing of the IgH enhancer element from 2 lupus strains (MRL-*lpr*, BXSB) revealed only a single point mutation at the non-core region of the MR-*lpr* enhancer compared to the BALB/c IgH enhancer (data not shown), the significance of which is currently under investigation by transfection experiments.

Autoantibody-Specific Genetic Elements – If, as these data suggest, the Ig-V_H loci in lupus mice are indeed normal or, alternatively, that germline genes encoding autoantibodies are present in both autoimmune and normal inviduals, then what might be the basis of autoantibody formation? One possibility is that are generated by qualitative defects in the somatic mechanisms creating antibody diversity, namely, rearrangement, H/L chain pairing, and somatic mutation. In other words, while it appears that the same Ig-V gene options are available to both normal and lupus mice, it might still be possible that autoantibodies are derived from an abnormal selection mechanism of the V or D gene segments, resulting in Igs unique to lupus mice. To test this possibility and define the contribution of germline genes and of somatic events in the generation of autoantibodies, we undertook identification and analysis of the genetic elements encoding hybridoma-derived monoclonal autoantibodies from various mice with spontaneous or induced autoimmunity. In this way, the effects of the genetic background and means of induction in the genetic elements coding for autoantibodies could be examined, and correlations between autoantibody structure and pathogenicity could be drawn.

The genetic elements encoding 10 autoantibodies that we have cloned and sequenced thus far are listed in Table I (Kofler et al., 1985a; 1987, and unpublished observations). The monoclonals were derived from MRL-*lpr* or NZBxW mice and had specificities for DNA, histones or IgG. Most were of the IgM isotype, but one IgG2a anti-DNA and one IgG3 anti-histone were included. Our data thus far indicate that a large number of genetic elements might be involved in autoantibody production. Thus, the 10 autoantibody H chain V regions employed 8 different D segments and all known J_H segments. The 8 L chains analyzed used V_k genes from 6 different V_k subgroups and all known functional J_k segments. Furthermore, as shown in Table II, although the 9 of 10 autoantibodies were encoded by V_H genes belonging in the large J558 family, the V_H genes for 7 of them were demonstrably different. In addition, it appears that other V_H gene families participate in the murine autoimmune response (Painter et al., 1986; Eilat et al., 1984; Arant et al., 1986; Shlomchik et al., 1986; Aguado et al., 1987; Naparstek et al., 1986).

The data support the concept that murine lupus-associated autoantibodies derive from a large number of clonally unrelated B cells that utilize the same or similar germline repertoire as non-self directed B cells. Self-specificity, therefore, may either result from certain combinations of V/D/J germline segments and/or complete H and L chains, or from somatic mutations of antibodies originally directed against exogenous antigens. These possibilities, in particular whether autoantibodies are generated by unmutated germline genes or require productive somatic mutations, are the subject of future analyses employing appropriate germline gene cloning, transfection and site-specific mutagenesis experiments. It should be noted, however, that our studies have not excluded the possibility that certain idiotypes predominate at certain time-points in the ontogeny of lupus mice or whether specific idiotypes may play a predominant role in disease pathogenesis.

T Cell Antigen Recptor Genes in Autoimmune Mice – Since T cells are essential for the development of murine SLE, we have also analyzed autoimmune T cell populations for possible defects at genetic loci coding for the T cell antigen receptor (TCR), the molecular

TABLE I

Genetic elements encoding murine lupus-associated autoantibodies

Monoclonal Ab code	Strain	Specificity	Isotype	Heavy Chain V_H	Heavy Chain D	Heavy Chain J_H	Light Chain V_k	Light Chain J_k
BxW-DNA7	BxW	ssDNA	IgM	J558	DFL16.1	J_H1	n.d.*	n.d.
BxW-DNA14	BxW	ssDNA	IgM	J558	DFL16.1	J_H2	V_k2	J_k1
BxW-DNA16	BxW	ssDNA	IgM	J558	DFL16.1	J_H2	V_k9	J_k2
MRL-DNA10	MRL/1	ssDNA	IgM	J558	n.a.**	J_H3	V_k1	J_k4
MRL-DNA4	MRL/1	ssDNA	IgG2a	V_H10^+	n.a.	J_H3	V_k1	J_k2
MRL-DNA22	MRL/1	ssDNA, dsDNA	IgM	J558	n.a.	J_H4	V_k5	J_k4
MRL-RF7	MRL/1	IgG2b, ssDNA	IgM	J558	n.a.	J_H1	n.d.	n.d.
MRL-RF14/9	MRL/1	IgG2a	IgM	J558	n.a.	J_H4	V_k28	J_k1
MRL-RF24	MRL/1	IgG1, 2a, 3	IgM	J558	Q52	J_H4	V_k29^+	J_k1
MRL-Histone7	MRL/1	Histones	IgG3	J558	SP2	J_H4	V_k5	J_k5

* n.d. = not determined

** n.a. = not assigned

+ The MRL-DNA4 V_H gene and the MRL-RF24 V_k gene could not be assigned to any known V_H or V_k gene family. They may represent the prototypes of new families (provisionally termed V_H10 and V_k29, respectively).

TABLE II

Percent homology of autoantibody V_H regions*

	BxW DNA7	BxW DNA14	BxW DNA16	MRL DNA10 and 22	MRL RF7	MRL RF14/9	MRL RF24	MRL Histone 7
BxW-DNA7	100	–	–	–	–	–	–	–
BxW-DNA14	85.8	100	–	–	–	–	–	–
BxW-DNA16	83.5	84.5	100	–	–	–	–	–
MRL-DNA10 and MRL-DNA22**	83.7	89.0	83.2	100	–	–	–	–
MRL-RF7	79.4	79.0	76.5	79.3	100	–	–	–
MRL-RF14/9	71.3	75.8	74.1	73.8	71.9	100	–	–
MRL-RF24	81.8	79.8	78.0	80.9	98.5	72.0	100	–
MRL-Histone7	76.0	77.2	75.3	77.3	72.3	92.7	72.8	100

*Complete V_H gene encoded sequences (including 5' untranslated region) of the indicated autoantibodies were compared, with the exception of BxW-DNA 7 and 14, where sequences start at codon +13 and +26, respectively.

**MRL-DNA 10 and 22 express identical V_H genes.

structure of which has recently been identified (reviewed in Kronenberg & Hunkapiller, 1985). TCR molecules are composed of α and β chains which are divided into V and C regions. Like Ig genes, the β chain genes are divided into separate Vβ, Dβ, and Jβ gene segments that are assembled by recombination during T cell development to form a Vβ gene associated with either of 2 constant (Cβ1 and Cβ2) genes. There are 6 functional Jβ gene segments clustered just upstream of each Cβ gene, and 2 Dβ gene segments. It is broadly agreed that the Vβ segments are relatively few (perhaps 20-30) in number, and usually unique (single-member gene subfamilies) except Vβ8 and Vβ5, composed of 3 and 2 functional members, respectively. There is no evidence as yet that either Cβ region is used preferentially in specific T cell subsets, since different functional helper and cytotoxic T cell lines have been found to randomly express T cell receptors of either the Cβ1 or Cβ2 type. The α chain genes have similar

organization, but differ from the β chain genes in that they contain a single Cα region gene segment and a much larger Jα gene segment number (perhaps larger than 50). Furthermore, the Vα repertoire appears to be much larger than Vβ repertoire, with the 11 Vα subfamilies identified thus far consisting of 1 to 10 members each. The primary means by which TCRs diversify appears to be by joining recombinations of the various genetic elements and not by somatic hypermutation which, unlike Ig molecules, is minimal. A third TCR gene, the so-called γ-chain gene, has also been identified, but its expression and function has not yet been fully defined.

a) *Genomic organization of Cα and Cβ T Cell Antigen Receptor Genes* — In an initial structural analysis of the TCR locus, we screened classical autoimmune and *lpr* homozygous autoimmune mice along with additional ancestral normal strains for gross alterations in TCR α and β genes (Noonan et al., 1986). Liver DNAs isolated from these mice were digested with restriction enzymes, electrophoretically size-separated on agarose gels, transferred to nitrocellulose, and hybridized with DNA probes complementary to either the α or β chain C region.

Hybridization of the Cα region probe to EcoRI digested DNA identified genomic DNA fragments (4.4 and 6.8 kb) encoding the Cα gene, but revealed no polymorphisms (Fig. 2A). Similar results were obtained with BamHI and Hind III digested genomic DNAs. Hybridization of BamHI digested genomic DNA with a TCR β chain proble capable of detecting Cβ1, Cβ2, Dβ2 and Jβ2 genes also disclosed monpolymorphic DNA fragments (6 and 10 kb) in 16 of the 18 strains tested (Fig. 2B). This high degree of conservation in the murine TCR C region locus contrasts with that found in humans where multiple restriction polymorphisms have been identified at both α and β chain loci (Robinson & Kindt, 1985; Hoover et al., 1985; Concannon et al., 1987). The two exceptions with the β gene probe were NZW mice, in which only one hybridizable band (9 kb) was detected, and (NZBxW) F_1 mice with the expected composite NZB and NZW profile (three bands of 6,9 and 10 kb), indicating the presence of both parental alleles.

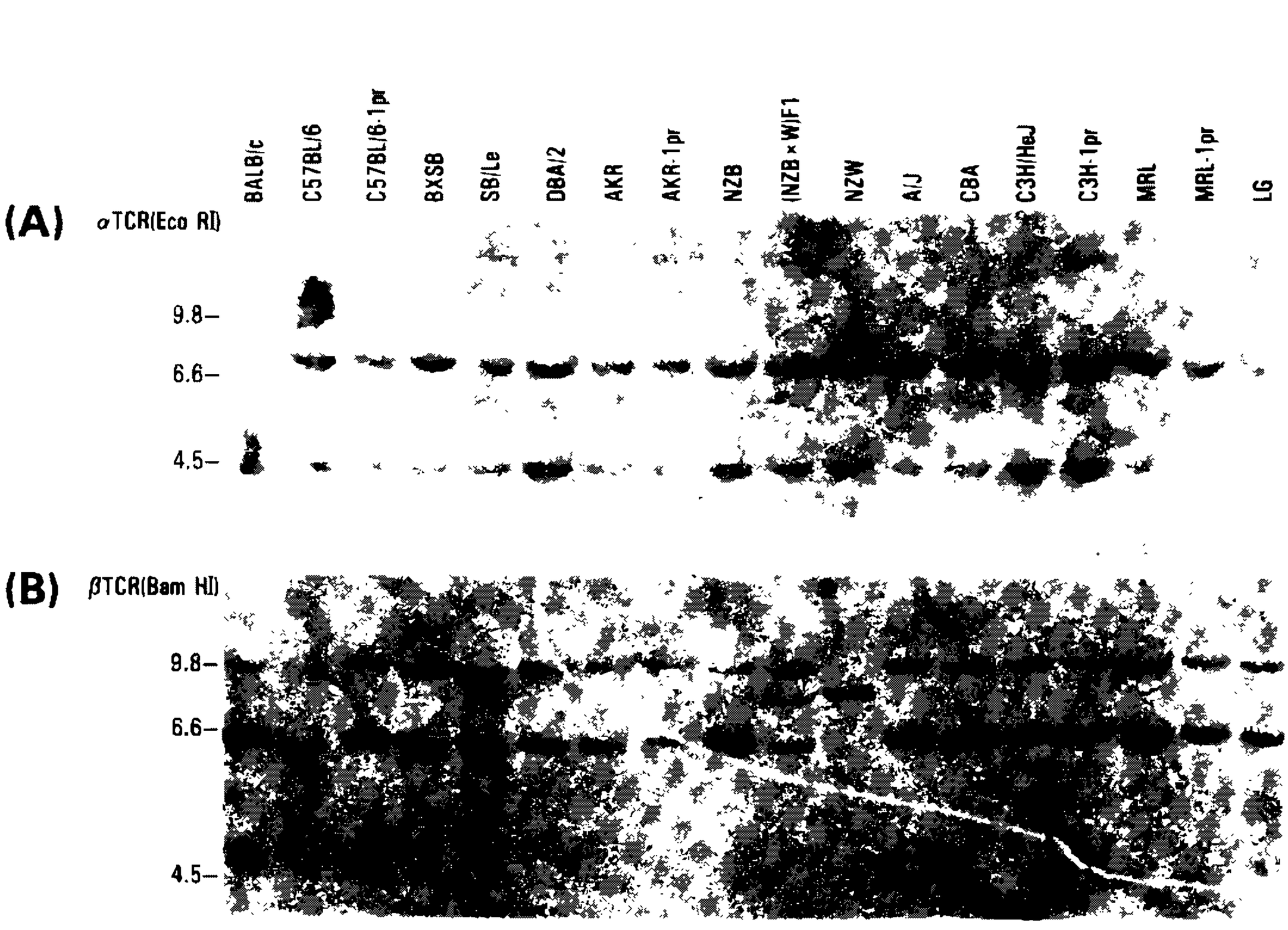

Fig. 2: RFLP analysis of TCR C genes of autoimmune, *lpr* congenic and normal background mice. Size-separated liver DNA digested with either EcoRI (A) or Bam HI (B) was probed with either an α chain constant region cDNA clone (A) or a β chain cDNA clone containing Cβ2, Jβ2, Dβ2 structures (B). Size markers (in Kb) (Hind III-digested γ DNA) are on the left side of each blot.

Considering the published restriction map for the TCR β-chain locus and additional digests, we conclude that the results with the NZW genomic digests were most consistent with the occurrence of a large (~8.8 kb) deletion beginning somewhere between the 2 EcoRI sites near the Cβ1 gene and extending downstream to somewhere between the ´2 Hind III sites flanking the Cβ2 gene.

To precisely define the NZW Cβ locus deletion, we cloned and sequenced the genomic fragment containing the NZW Cβ gene (Noonan et al., 1986). The intron/exon organization of the gene, sequencing strategy, and alignment of the NZW Cβ nucleotide sequence with the published Cβ1 and Cβ2 gene sequences are shown in Fig. 3.As indicated, the sequence 5' of exon 1 in the NZW gene was identical to Cβ1, but not Cβ2, whereas the sequence 3' of exon 1 (in the first intron) matched Cβ2. Two polymorphic residues identified within exon 1 of the Cβ gene, one at position 261 (C in Cβ1, A in Cβ2) and another at position 149 (A in Cβ1 and in several reported Cβ2 sequences, G in one Cβ2 cDNA sequence) could be used to further map the NZW deletion. The NZW Cβ sequence corresponded to Cβ2 in both these positions, thus placing the origin of the deleted region within the first 167 base pairs of exon 1 of the Cβ1 gene (residues-18 to 149) and extending to the complementary region within the first exon of the Cβ2 gene.

Therefore, a meiotic recombination event can be invoked to explain the deletion with unequal crossing over between the precisely aligned first exons of the Cβ1 gene on one chromatid and Cβ2 gene on its homolog, producing a Cβ locus lacking Cβ1, Dβ2 and Jβ2 regions.

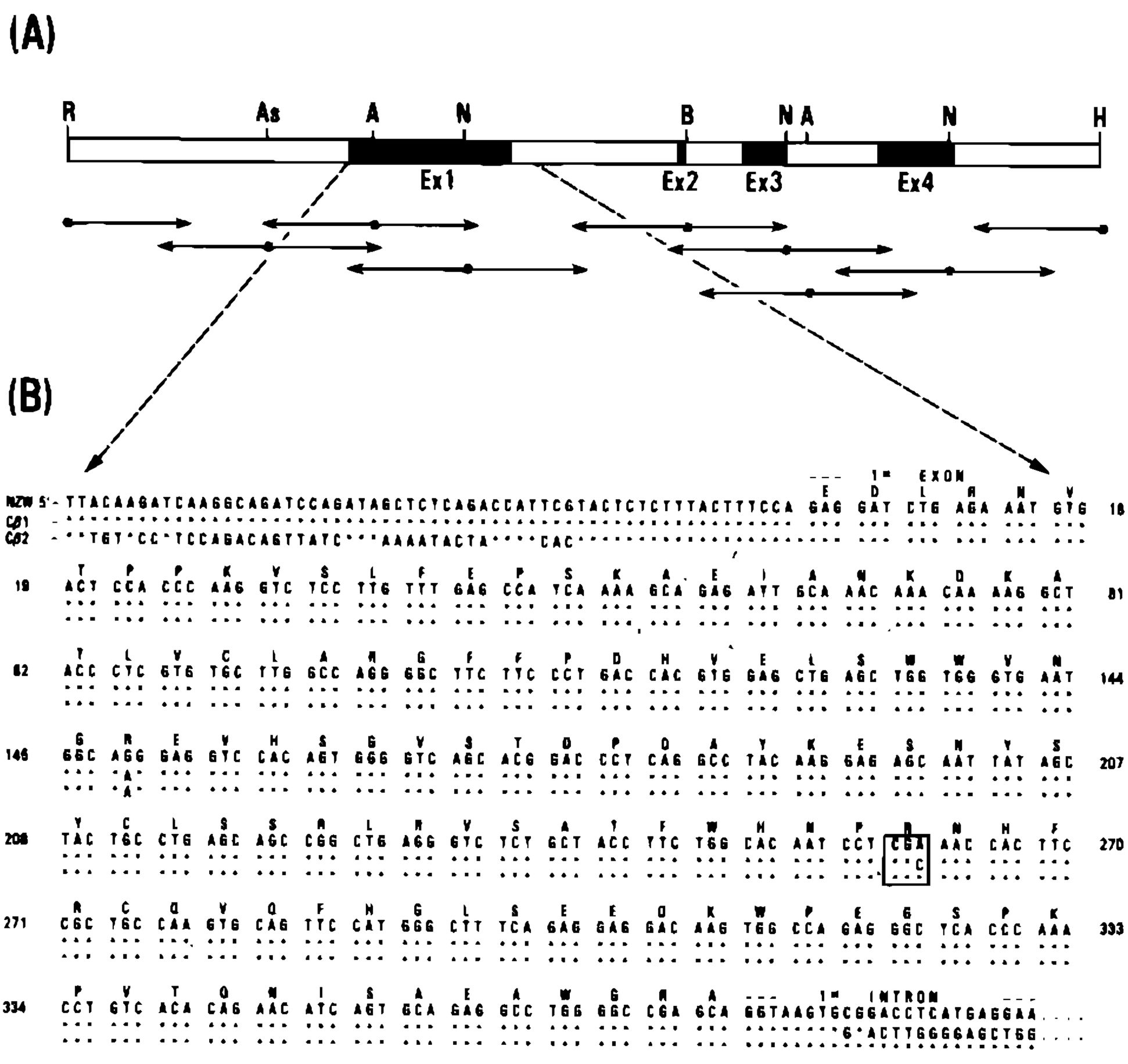

Fig. 3: Sequencing strategy (A) and comparison of relevant first exon NZW sequence with that published for Cβ1 and Cβ2 genes. The intron (open lines)/exon (shaded lines) conformation of the NZW Cβ locus along with specific restriction sites used in sequencing analysis appear in 5A. In 5B is a single 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 = EcoRI; As = ASP 718; A = Ava II; N = Nco I; B = Bgl II; H = Hind III; Ex1 = Exon 1.

Based on Southern blot analysis only, Kotzin et al., (1985) independently derived similar conclusions. Due to this deletion, only Dβ1-Jβ1, and not Dβ2-Jβ2 and Dβ1-Jβ2, joinings are possible in NZW mice; thus these mice may be able to generate only one third of the TCR β-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, since these mice exhibit near normal humoral and cellular responses studied thus far (Theofilopoulos et al., 1985). The significance of the NZW TCR β-chain deletion to autoimmunity remains to be determined. Although the longevity of these mice is near normal, late in life they develop various autoantibodies and contribute significantly to the early-life severe lupus of the classical SLE hybrid (NZBxW) F_1, as well as in hybrids with other SLE-prone strains. To date, however, our studies with (NZBxW) F_2 mice have not desclosed any primary role of this deletion in the development of autoimmune manifestations (unpublished observations).

b) *Genomic organization of TCR Vβ genes* – The organization of the V gene loci encoding the antigen-specific TCR in autoimmune mice is *a priori* of considerable interest that has been further stimulated by the recent description of mice (SWR, SJL, C57L, C57BR) with a genotype lacking up to 50% of the normal TCR Vβ gene repertoire (Behlke et al., 1986). These mice have been shown to have immunologic abnormalities including high susceptibility to induction of experimental autoimmunity. Using probes isolated in our laboratory, we have begun to investigate the organization of TCR Vβ genes in autoimmune mice. Southern blots of genomic DNA from all common autoimmune strains and their normal ancestors as well as from *lpr* congenic strains were prepared and hybridized with Vβ probes. Results thus far, using 6 different restriction enzymes and several Vβ genes (Vβ1, 2, 6-15, and 18), have indicated no RFLPs between abnormal and normal mice, nor lack of any of the tested Vβ genes (Singer et al., in preparation). Further experiments are underway to test additional Vβ genes.

c) *Genomic organization of Vα genes* – The genomic organization of Vα genes has also been analyzed, and the following conclusions have been reached (Singer et al., in preparation): 1) Vα subfamily probes, in contrast to Vβ subfamily probes (see above), revealed several EcoRI RFLPs among autoimmune and normal strains. Based on EcoRI restriction patterns, four distinct TCR Vα genotypes could be distinguished among the strains tested. 2) The *lpr* autoimmunity accelerating/inducing gene did not affect Vα (or Vβ) gene RFLPs on any genetic background. 3) Autoimmune BXSB mice derived their Vα locus from the ancestral C57BL/6 strain and not the SB/Le ancestral strain, even though the SB/Le is considered to be the primary contributor to autoimmunity in BXSB mice. 4) Autoimmune NZB mice have the same Vα genotype as SJL and SWR mice, whereas the NZW mice have a Vα genotype unique among all murine strains tested. In the NZW genome, both deleted and extra Vα genes appear to be present. NZB and NZW mice contribute to the (NZBxW) F_1 and (NZBxSWR) F_1 disease, and SJL and SWR mice are known to be susceptible to autoimmune disease induction. The above findings suggest that particular constellations of Vα-Vβ genes (possibly together with particular MHC haplotypes) might predispose to autoimmune disease.

d) *TCR Vβ gene expression in enlarged MRL-lpr/lpr lymph nodes* The *lpr* gene accelerates an SLE-like disease in predisposed background mice and induces autoantibodies in mice of normal genetic background. The molecular mode(s) of its action and chromosomal location remain unknown, but it is primarily expressed as a massive T cell proliferation manifested only in the presence of a thymus. The proliferating cells ($Thy1^+$, dull $Ly1^+$, $Lyt2^-$, $L3T4^-$, $B220^+$) have many peculiar phenotypic characteristics that complicate their classification with regard to lineage, origin and stage of differentiation. Nevertheless, on the basis of certain dominat characteristics such as thymic dependence, lack of Ig gene rearrangement (Morse et al., 1982) and increased c-*myb* mRNA levels (Mountz et al., 1984), these cells have been tentatively classified into a relatively immature T cell lineage. Recently, Nemazee et al. (1985) demonstrated that *lpr* lymph node cells express TCR α-and β-chain mRNA, and that the TCR gene rearrangement pattern detected by Southern hybridization excludes monoclonal or limited oligoconal T cell expansion. Further knowledge on the clonal make-up of the expanded lymph node population may be important to understand the etiology of the *lpr* phenomenon and the role of *lpr* cells in accelerating autoimmunity.

To analyze Vβ gene expression and clonal diversity in MRL-*lpr*/*lpr* lymph node cells, we

screened an unamplified cDNA library prepared from such cells (Singer et al., 1986). We isolated 47 Cβ-positive clones of 100,000 screened, transferred their cDNA inserts to M13mpll, and perfomed dideoxy sequence analysis using an oligonucleotide primer hyridizing near the C-J junction. This allowed rapid determination of the complete J- and D-region sequences and more than 200 bases of V-region sequences from each of our clones. The relevant sequence data from 27 of the Cβ-positive clones in which informative rearrangements had occurred are shown in codon register in Table III. Clearly, the rearrangements are all in the correct reading frame to yield translatable β-chain mRNAs. Significantly, every β-chain cDNA sequence was derived from a unique rearrangement with specific D and J elements used in an apparently random fashion. We conclude from these results that the TCR β-chain mRNA population, and hence the underlying clonal makeup, of the abnormally proliferating lymph node T cells in MRL-*lpr/lpr* mice is extremely heterogeneous with a Vβ gene pool size of at least 500. This represents only a minimum estimate of the β-chain diversity since no duplicate sequence were found.

TABLE III

T cell receptor VDJ joining regions of MRL-*lpr/lpr* lymph node cDNA clones

Clone No.		V gene*	J element
		*I. Dβ1.1 Rearrangements***	
M*lpr*	2	18+	*GA CAG* A	TT TCC (1.4)
	4	8.3	GA *GAC AGG GA*	C AAC (1.4)
	6	8.3	GA *GGG GAC AC*	C TAT (2.1)
	9	7	TA GAC T*GG ACA GGG G*	CT GGA (1.3)
	14	14	TT TAC CC*G GGA CAG GA*	A AAC (1.1)
	17	8.3	GT *GAC AGG GG*	A AAC (1.1)
	31	6	CC *GGA CAG* GTA C	CC AAC (1.4)
	50	7	CC *GAC AGG GGA* C	AC ACC (2.4)
	51	9	GG TC *G GGA CA* A AG	C TCC (2.6)
	32	8.3	CC CC*G GGA CAG GGG G*	CT GGA (1.3)
	56	8.2	AT C*GG GAC AGG GA*A	TAT (2.1)
		II. Dβ2.1 Rearragements	
M*lpr*	3	8.2	AT GCC A*AC TGG GGG* CGG G	AT GAA (2.6)
	11	6	GT T*TG GGG* CC	C TCC (2.6)
	15	10	*CG ACT GGG GGG GC*G C	AC ACC (2.5)
	20	14	TA *GGA CTG GGG G*	GT CAA (2.4)
	23	8.3	GT GAT GT*C TGG GG*T	AGT (2.3)
	34	10	A*G ACT GGG GG*	T AAC (2.1)
	35	8.3	GT GAT GCA CG*T GGG GGA*	GCA (2.3)
	39	8.3	GT GAT GG*C TGG GGG G*	CC TAT (2.6)
	45	8.2	AT *GGG ACT GGG GGG* C	GT CAA (2.4)
	59	8.2	AT GCG *TGG GGG GG*G	AGT (2.4)
	26++		....ACATTGT*GGGACTGGGCCCTC*	TAT (2.1)
	27++		....ACATTGT*GGGACTGGGGGGGCCCTCT*	GAA (2.6)
		III. Possible V-J Rearrangements	
M*lpr*	7	8.3	GT GAT GGC CAA	TAT (1.6)
	21	8.3	GT GAT C	CC AAC (1.4)
	37	8.3	GT GAT GCC	AGT (2.3)
	49	8.2	GG AGG	TCC (2.6)

* Nomenclature according to Behlke et. al. (36).

** Sequences that could be derived from genomic D elements are in italic.

\+ Provisional, following an additional Vβ gene reported by Kappler et al. (46).

++ Dβ2. 1 upstream flanking genomic sequences.

A notable finding from the data presented in Table IV was the apparent over-representation of Vβ8.2/Vβ8.3 genes (15/25 or 60% of the clones) in the MRL-*lpr/lpr* lymph node cDNA library. Interestingly, a third equally homologous member of this subfamily, Vβ8.1, despite its presence in the MRL-*lpr/lpr* genome as revealed by Southern blot analysis, was not found at all among the clones analyzed. Among the remaining 10 clones, we found 5 previously characterized (Vβ6, 7, 9, 10 and 14) and 1 putative new Vβ gene (Vβ18) with no single gene represented in more than 2/25 (9%) of the clones. Although information on the relative expression of individual Vβ genes in murine lymphoid tissues is somewhat limited, (Barth et al., 1985; Born et al., 1985; Patten et al., 1984) the enhanced representation of Vβ8 subfamily genes appears to be unique to the *lpr*-induced abnormal T cell subset. Paradoxically, with respect to our analysis of cDNA clones, 2 monoclonal antibodies (KJ16-133 and F23.1) which react with a TCR epitope that correlates with expression of Vβ8 subfamily genes (Behlke et al., 1986, 1987), stained only 17-20% of MRL-*lpr/lpr* lymph node cells. This rather low percentage of antibody-binding cells, despite the high proportion of Vβ8 clones in our cDNA library, can be explained by either: a) higher β-chain mRNA levels in Vβ8-expressors than in cells expressing other Vβ genes in the *lpr* lymph nodes, b) little or no staining of *lpr* cells expressing one of the Vβ8 subfamily genes, perhaps due to lower affinity of the monoclonal antibodies and/or lower density of receptors, or c) differential reactivity of the above monoclonals with members of the Vβ8 gene family (Behlke et al., 1987). Abnormalities in expression of the α-chain, or accessory molecules like the T3 complex, could cause such decreased receptor density. *In situ* hybridization or mRNA protection analysis will be required to distinguish between the above possibilities.

The high expression of Vβ8.2/8.3 genes in MRL-*lpr/lpr* lymph node cells may indicate a) selective activation, perhaps based on a common structural feature or specificity encoded by these genes; b) ontogenic preference for rearrangement of certain Vβ genes over others, perhaps based on Cβ locus proximity, as recently suggested for Ig genes (Perlmutter et al., 1985), and/or c) *lpr*-induced modification of the thymic selection/maturatrion process that allows exportation in the periphery of Vβ8.2/8.3 gene expressing cells. The generality of this skewed Vβ gene expression and its possible implications for lupus awaits analysis of additional strains, particularly *lpr* homozygous mice of different genetic backgrounds.

TABLE IV

Distribution of T cell receptor Vβ genes expressed by MRL-*lpr/lpr* lymph node cDNA clones

Expressed Vβ Genes	Isolates from unamplified library, no.
Vβ6	2
Vβ7	2
Vβ8.2	5
Vβ8.3	10
Vβ9	1
Vβ10	2
Vβ14	2
Vβ18	1

CONCLUSIONS

In summary, a number of different strains of mice spontaneously develop an autoimmune disorder with features of human lupus. The clinical syndromes of these mice vary significantly, as do their serologic, cellular and genetic abnormalities. The basis(es) for this syndrome is not yet defined, but lymphoid cells of such mice are sufficient to carry the information necessary to transmit the disease.

The most important immunologic pertubation in murine (and human) lupus is a B lymphocyte hyperactivity with corresponding enhancement of serum antibodies and autoantibodies, and consequent formation of pathogenic antigen-antibody immune complexes. The presence of autoantibodies in 1) some recombinant lupus x normal murine lines expressing the normal partners' Ig-C allotypes, 2) normal mice in which exogenous (LPS) or endogenous (*lpr* gene) immunostimulators have been introduced, and 3) unmanipulated normal mice, all indicate that lupus mice are not unique in their Ig genetic elements. This conclusion has been verified by the fact that digested genomic DNAs of lupus mice showed similar RFLP patterns to those of normal mice when analyzed with probes corresponding to all known murine V_H gene families. Furthermore, nucleotide sequencing of a few autoantibody genes has demonstrated that autoantibodies are not encoded by unique genetic elements. It therefore seems likely that their specificity for self results from somatic mechanisms such as combinations of certain germline

genetic elements (V, D, J and of entire Ig chains) and/or somatic mutations.

The cause of B cell hyperactivity in lupus has not yet been elucidated, although autonomous B cell maturation does not appear likely since: 1) B cell proliferation/differentiation in lupus mice is dependent on accessory T cell derived signals; 2) helper T cell deletion *in vivo* with appropriate antisera abrogates autoimmunity, and 3) neonatal thymectomy prevents the MRL-*lpr* disease. RFLP analysis of digested genomic DNAs from lupus mice failed to disclose any structural abnormalities in α and β chain TCR C genes, except a deletion of Cβ1-Dβ2-Jβ2 genetic elements in the NZW genome, the importance of which remains to be explored. The genomic organization of TCR Vα and Vβ families has also been investigated. The results indicate that among classical lupus strains, there are no Vβ polymorphisms, but several Vα polymorphisms could be identified. The relevance of these polymorphisms in disease pathogenesis is currently being investigated in appropriate F_1 and F_2 mice. Cloning and sequencing of the MRL-*lpr* TCR Vβ genes established the polyclonal origin of the aberrantly proliferating T cells of these mice and, importantly, showed a significant bias in the representation of the Vβ8 family. This enhanced representation may indicate modifications in thymic selection/maturation processing in these mice which may have relevance to the *lpr* gene-associated autoimmunity. We are currently utilizing *in situ* hybridization, mRNA protection assays and Southern blot procedures to further analyze the molecular organization, expression and repertoire of T cell receptor V genes in lupus and other autoimmune and normal strains.

It is abundantly clear from the work to date on murine models that systemic autoimmune syndromes, although caused by genetically-determined lymphoid cell abnormalities, are extremely complex and appear to be initiated by several basic abnormalities and pathways. Over the last two years, we and others have re-directed our research into the molecular biology of these disorders. Although this type of research is relatively new, several important observations have emerged which provide new avenues of investigation and exclude certain possibilities from further follow-up. Understanding the molecular genetics of spontaneous murine models of systemic autoimmunity will significantly enhance our efforts to understand the respective human diseases.

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