Brief Definitive Report

Ig HEAVY CHAIN VARIABLE REGION GENE COMPLEX OF LUPUS MICE EXHIBITS NORMAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM

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The apparent central role of Ig hyperproduction, particularly the high levels of autoantibodies in murine and human lupus (reviewed in 1 and 2), suggest a possible contribution of abnormalities in structural and/or regulatory sequences of Ig genes in the pathogenesis of this disorder. To address this possibility, the Ig heavy chain variable region (Igh-V) gene complex in all major lupus strains and their nonautoimmune ancestors were subjected to restriction fragment length polymorphism (RFLP) analyses (3) using DNA probes corresponding to seven Igh-V gene families. These families comprise the majority of the known polymorphic murine V_H gene germline repertoire (4), including some encoding several autoantibodies, as shown by complementary DNA (cDNA) (5, and our unpublished observations) and protein (6) sequencing. Our experiments allowed Igh-V haplotype assignment for the respective strains, and showed that the Igh-V loci of lupus and Igh-V haplotype–matched nonlupus mice result in essentially identical restriction fragment patterns. This suggests that the Igh-V gene complex in lupus mice was inherited basically unaltered from their nonautoimmune ancestors, and therefore probably does not carry a primary defect responsible for autoantibody production in this disease.

Materials and Methods

The generation of the BXSB, MRL (MRL/n and MRL-*lpr* lines), NZB, and NZB × W F_1 lupus mice, and of *lpr* congenic strains of normal mice has been described (1, 2). High molecular weight DNA was prepared as previously described (7). Briefly, frozen livers were treated with proteinase K, extracted with phenol/chloroform, dialyzed, and treated with pancreatic RNase A. The V_H gene probes used were derived from cloned DNA inserts cleaved from their respective vectors and labeled by nick-translation with [^{32}P]-deoxynucleotide triphosphates (8). Their sources were: 7183 family; plasmid F9V21 (9) digested with Pvu I and Bgl II resulted in a fragment containing virtually the entire V_H

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coding region, diversity (D) and J_H4 (heavy chain joining region) segments of the MOPC21 heavy chain. Q52 family; the productive rearrangement of the QUPC-52 myeloma from codon 14 to the Hha I site in J_H1 was given to us by Drs. M. Shapiro and M. Weigert (Institute for Cancer Research, University of Pennsylvania, Philadelphia, PA). S107 family; a Pst I fragment containing sequences encoding part of the leader peptide and the entire V_H region up to the J_H1 Hha I site of the S107 myeloma (10) was used. J558 family; this family was probed with a Pst I fragment of the subcloned productively rearranged allele from myeloma J558 (4). X-24 family; Gal55.1 is a BALB/c germline V_H gene (11), encoding the exact V_H region of myeloma protein X-24. J606 family; another BALB/c germline V_H gene (V14A), cloned into pBR322 by Dr. S. Crews (Department of Pathology, Stanford University, Palo Alto, CA) was used. 36-60 family; an Eco RI/Ava II fragment, containing only V_H sequences from a dinitrophenyl-binding hybridoma antibody, was contributed by Drs. S. Riley and R. Ogata (Department of Molecular Biology, Scripps Clinic and Research Foundation).

RFLP analyses were performed essentially as described (3, 4). Briefly, liver DNA was digested with restriction enzyme, electrophoresed through 0.7% agarose, transferred to nitrocellulose, and prehybridized. Hybridization was performed for 16–24 h, after which the blots were washed and autoradiographed.

Results

Autoradiographs of restriction enzyme–digested, size-separated, genomic DNA from lupus mice, their ancestors, and other nonautoimmune strains probed with ^{32}P-labeled DNA corresponding to seven murine V_H gene families are shown in Fig. 1, *A–C* (for brevity, only a representative autoradiograph for each family corresponding to one of three enzymes is depicted). All autoimmune mice revealed restriction fragment patterns identical to those of their nonautoimmune ancestors or, in case of unknown derivation, closely resembling those of normal strains. MRL mice, which are descendents from LG (75%), AKR (13%), C3H (12%), and C57BL/6 (0.3%) progenitors (1, 2), shared identical patterns with C3H/HeJ and LG. BXSB mice completely matched their two ancestors, C57BL/6 and SB/Le. The New Zealand (NZ) strains, the derivations of which are unknown, showed identical restriction fragment patterns with the AKR strain in six V_H families, and differed only by a single band in the Q52 family (indicated by arrows in the respective autoradiograph in Fig. 1 *C*). Furthermore, introduction of the *lpr* gene, associated with excessive Ig levels and high titers of autoantibodies (2), did not alter the restriction fragment patterns compared to those of the non-*lpr* parental background strains.

In addition, these data permitted Igh-V haplotype assignments for all major lupus and several other inbred strains of mice, based on comparison of their

TABLE I
Igh-V Haplotype Assignment

Igh-V Haplotype	Strains
Igh-V^a	BALB/c*
Igh-V^b	C57BL/6*, C57BL/6-*lpr*, BXSB, SB/Le
Igh-V^c	DBA/2*
Igh-V^d	AKR*, AKR-*lpr*, NZB*, NZB × W F_1, NZW
Igh-V^e	A/J*
Igh-V^j	CBA/J, C3H/HeJ*, C3H-*lpr*, MRL-n, MRL-*lpr*, LG

Igh-V haplotypes were assigned based on comparisons of restriction fragment patterns from previously typed reference strains (4) (indicated by asterisks) included in this study.

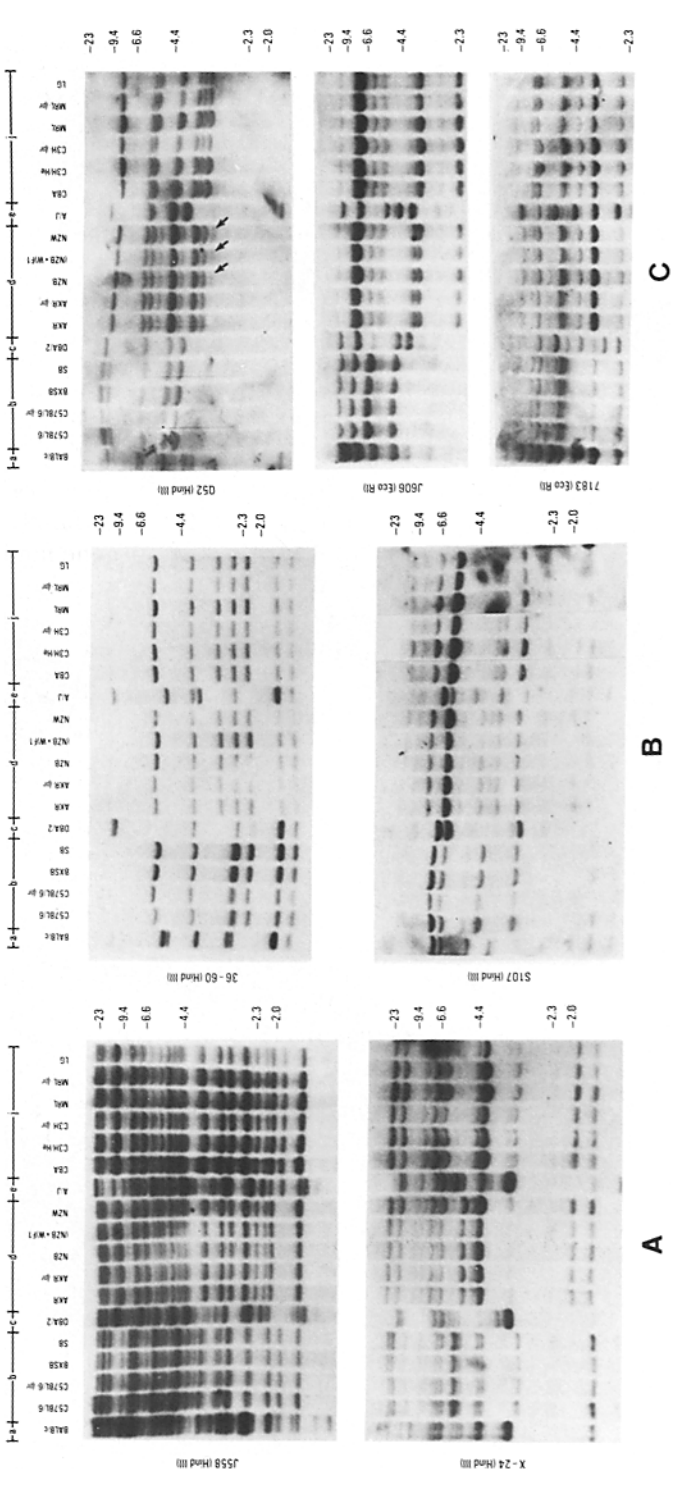

FIGURE 1. Autoradiographs of restriction enzyme–digested, size-separated liver DNA from several lupus and other inbred strains of mice probed with ^{32}P-labeled DNA corresponding to seven murine V_H gene families. The strains have been organized according to their Igh-V haplotypes (indicated by lower 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 autoradiograph 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.

restriction fragment patterns with those of previously typed reference strains (4) (Table I). Our results place BXSB, SB/Le, and C57BL/6-*lpr* in Igh-V^b, the NZ mice and AKR-*lpr* in Igh-V^d, and the MRL strains, LG and C3H-*lpr*, in Igh-V^j haplotypes.

Discussion

The central question we addressed was whether lupus mice have acquired structural alterations in their Igh-V loci that might contribute to autoantibody expression and disease. For this purpose, genomic DNA from lupus mice, their ancestors, and other inbred strains, was examined for RFLP using labeled DNA probes corresponding to prototype members of seven murine V_H gene families (4). No difference was observed between MRL or BXSB lupus mice and their Igh-V haplotype–matched ancestors, or other non-autoimmune strains, using three different restriction enzymes. The unknown derivation of NZ mice precluded comparison with their direct ancestors. Nevertheless, the Igh-V haplotype identified in these mice was indistinguishable from the Igh-V^d pattern of strain AKR, with the exception of an additional fragment in the Q52 family. This difference probably reflects the normal rate of divergence of V_H gene families in murine species. A significant contribution to autoantibody generation by a single V_H gene that might correspond to this fragment is unlikely, since lupus-associated autoantibodies probably derive from a number of V_H genes from different families (5, 6, and our unpublished observations). Introduction of the *lpr* gene into normal mice, which causes lymphoproliferation and expression of high titers of autoantibodies (2), had no detectable effect on the Igh-V gene complex.

This study also allowed Igh-V haplotype assignments based on comparisons of unknown strains with previously classified prototypes (4). All strains tested revealed haplotypes with no evidence of recombination within the Igh-V locus. In instances where the (serologically determined) Ig heavy chain constant region (Igh-C) haplotype was known, it correlated well with the Igh-V haplotype. In the NZ strains (Igh-C^n), whose Igh-C gene complex resulted from a recombination between Igh^d and Igh^e (12, 13), the Igh-V haplotype corresponded to that of the 5′ portion of the Igh-C gene complex (Igh^d). We found MRL mice, which have been typed serologically as *a* in the C region (1), to be *j* in the V region, suggesting a recombination in this strain as well. However, the serologic data must be questioned, since the Igh^j haplotype was unknown at the time of typing, and Igh^j mice, including the prototype strain CBA/J, have been previously classified serologically as Igh^a (14). Additional RFLP analyses using DNA probes corresponding to the C-μ and C-α switch regions indicate that the MRL Igh-C gene complex is *j* and not *a*.[1] Therefore, the MRL Igh gene complex probably derives directly from either LG or C3H, both Igh-V^j, without recombination.

Based upon, and hence within the limitations of RFLP analysis, our study suggests that the Igh-V gene complex in lupus mice may be essentially normal. This technique can reveal recombinations, gene loss or duplication, sequence alterations, and even single point mutations, provided these changes detectably

[1] Kofler, R., J. Gautsch, P. A. Tsonis, F. J. Dixon, and A. N. Theofilopoulos. Analysis of restriction fragment length polymorphism in the immunoglobulin heavy chain constant region gene complex of lupus mice (manuscript in preparation).

alter the length of restriction fragments hybridizing to the labeled DNA probes. Differences that do not fulfill these requirements will remain undetected, and yet might contribute to the serological 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 (5, 6 and our unpublished observations). 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 seven DNA probes, recognizing most V_H genes within this relatively small chromosomal region, were used. Our conclusion is further supported by classical genetic studies on NZB lupus mice indicating that the production of autoantibodies and disease development do not segregate with V_H genes from autoimmune mice (15, 16). Therefore, the existing evidence from classical genetic studies and RFLP analyses strongly suggests that the Igh-V gene complex is not causally involved in murine lupus.

If, as suggested by current data, the Ig loci in lupus mice are indeed normal, self-specificity in lupus could be generated by qualitative or quantitative defects in the somatic mechanisms creating antibody diversity, namely rearrangement, heavy/light chain pairing, and somatic mutation. Alternatively, self-specific B cell clones could be a normal part of the immune system, perhaps a by-product of somatic diversification. The mechanisms controlling such potentially harmful clones under normal conditions might be inadequate in the face of the polyclonal B cell hyperactivity characteristic of murine SLE (1).

Summary

B cell hyperactivity, hypergammaglobulinemia, and autoantibody expression, the hallmarks of systemic lupus erythematosus, might be associated with structural abnormalities within the Ig heavy chain variable region (Igh-V) gene complex. The Igh-V loci from several lupus-prone mouse strains, their ancestors, and other nonautoimmune mice were therefore analyzed by restriction fragment length polymorphisms with DNA probes corresponding to seven V_H gene families. These seven families comprise the majority of the known polymorphic murine V_H gene repertoire, including some involved in autoantibody generation. Our study showed that the Igh-V loci from lupus and haplotype-matched nonlupus mice resulted in essentially identical restriction fragment patterns, a finding which suggests that the Igh-V gene complex does not carry a primary defect responsible for autoimmune disease.

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