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Research Article

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Molecular Analysis of the Human Immunoglobulin VλII Gene Family

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Abstract

The 8.12 idiotype characterizes a subpopulation of anti-DNA antibodies in patients with systemic lupus erythematosus (SLE). The idiotype is present on lambda light chains and has previously been shown to be exclusively encoded by VλII light chains. RFLP analysis of the VλII gene family has shown the family to consist of 10 to 15 members. Thus far, the sequences of seven VλII germline genes are reported in the literature with one of these a pseudogene. To identify the VλII genes that encode 8.12 positive antibodies and to further characterize the VλII family, germline VλII clones were derived from a patient with SLE. Two libraries were constructed: a genomic DNA library and a library of PCR-derived VλII gene products obtained using a conserved VλII leader region primer and a primer for the nonamer region 3′ of the coding sequence. We now describe seven new germline genes, two of which are pseudogenes. Comparison of VλII germline genes to sequences of 8.12 positive light chains produced by EBV-transformed B cell lines show that all 8.12 positive light chains are encoded by a limited number of highly homologous members of the VλII family. 8.12 negative VλII encoded light chains also derive from a limited number of VλII genes, suggesting that only a subset of the apparently available VλII genes are commonly expressed. (*J. Clin. Invest.* 1994. 94:532–538.) Key words: germline genes • lambda chain • 8.12 idiotype • somatic mutation • systemic lupus erythematosus

Introduction

Studies of murine anti-double stranded(ds)DNA antibodies have suggested that these autoantibodies are encoded by a restricted number of germline variable (V) region genes (for review see reference 1). The expressed V region genes display somatic mutations and the analysis of these mutations has led some investigators to conclude that the anti-DNA response is antigen driven and DNA is likely to be the selecting antigen (1, 2).

Studies of human anti-DNA antibodies have been more difficult. The generation of human IgG producing B cell lines remains a rate limiting step in the analysis of the expressed human immunoglobulin gene repertoire. In addition, the relative paucity, until quite recently, of human germline V gene sequences has often made the analysis of somatic mutation speculative.

We began our studies of anti-DNA antibodies through an idiotypic analysis of the serum anti-DNA response. By generating monoclonal anti-idiotypes to serum anti-DNA antibodies, we were able to define distinct subpopulations of anti-DNA antibodies. One such subpopulation consists of antibodies bearing the 8.12 idiotype (3, 4). This idiotypic determinant is present on lambda light chains. About 50% of patients with systemic lupus (SLE) have elevated titers of 8.12 positive antibodies. These antibodies can constitute as much as one half of a patient's anti-DNA response. In addition, 8.12 positive antibodies are present in glomeruli of patients with SLE, demonstrating that the idiotype is present in pathogenic anti-DNA antibodies (5).

We previously generated EBV-transformed B cell lines and showed that all 8.12 positive light chains are encoded by members of the VλII gene family (6). In our laboratory, analysis of the VλII gene family demonstrated that it consists of 10 to 15 members (7). We have now obtained germline VλII gene segments to extend the analysis of a V gene family which participates significantly in the anti-DNA response in SLE and to determine which members of this family encode 8.12 positive light chains.

Methods

Construction of a genomic library VλII genes. Genomic DNA was obtained from the spleen of SLE patient K.S. (8). The DNA was partially digested with MboI, ligated into EMBL three arms (Stratagene, La Jolla, CA) and packaged according to the manufacturer's instructions. The library (1×10^6 recombinant phages) was plated without amplification and screened for hybridization with a 255-bp HinfI/PstI VλII V region probe described previously (7). The probe was radiolabeled by nick translation and blots were hybridized at 42°C in 50% formamide and washed at 68°C in 2× SSC, 0.1% SDS.

V gene segments from phage clones 16.2 and 16.3 were subcloned into pBluescript II SK(+/−) (Stratagene) and were amplified by PCR using a 5′ oligonucleotide primer (5′ ATGGCCTGGGCTCTGCTC 3′) derived from the 5′ leader region of two VλII genes, VλII.1 germline gene (9), and DSC, a rearranged gene (7) which closely resemble each other and a 3′ primer (5′AAGGCCCTGGGCAGGTTTTGGT 3′) constructed from the 3′ nonamer recombination signal sequence (RSS) of the VλII.1 gene. PCR was performed as follows: one cycle of 5 min at 94°C followed by 30 cycles of 1 min at 94°C, 90 s at 55°C; 2 min at 72°C. The PCR products were cloned into the pCR™II vector using the TA cloning system, version 1.3 from Invitrogen Corp. (San Diego, CA). The V gene segment from phage clone 15.2 was obtained by direct PCR amplification.

Construction of a PCR amplification library. 1 μg of K.S. genomic DNA was amplified with 5 U of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Norwalk, CT) in a DNA thermal cycler (Perkin-Elmer Corp.) with the same conserved primers used to isolate VλII germline gene segments from phage clones as described above. 33 cycles of amplification consisting of 1 min at 94°C, followed by 2 min at 60°C, and 3 min at 72°C were performed. 10 μl of the reaction product was

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digested with EcoRI and electrophoresed in 2% agarose/TBE to confirm the presence of an appropriate sized product. PCR products were cloned using the TA cloning system as above. Clones were designated as follows: hslv2, homo sapien lambda chain variable subgroup 2, clone number.

To determine whether these conditions would give rise to chimeric sequences, two sets of reactions, each containing two plasmids encoding VλII, (equimolar amounts, 10 ng), (*a*) hslv216.319 and hslv215.23 set and (*b*) hslv2046 and hslv2018 set, were combined in parallel and used as templates for PCR with the conserved primers under the same conditions as described for the PCR amplification library. The PCR products were cloned in the same TA cloning system used for the PCR library. A total of 60 clones (30 clones from each set) were sequenced, and none represented a chimeric sequence.

Nucleotide sequencing and gene analysis. The nucleotide sequences of the PCR-derived inserts were determined by the dideoxynucleotide chain termination method using the Sequenase Version 2.0 kit (U. S. Biochemical Corp., Cleveland, OH). T7 and SP6 promoter primers were used, as well as the specific VλII oligos used for PCR amplification. The University of Wisconsin Genetics Computer Group (GCG) program, version 7.3, was used for nucleotide and amino acid sequence analysis, including the analysis of isoelectric points.

Results

VλII germline genes obtained from a genomic library. VλII genes were isolated from a genomic library of an SLE patient, K.S., screened with the 255-bp DSC VλII probe (7). Four VλII genes were obtained of which one was clearly novel. From phage clone 16.2, a single VλII germline gene was identified, and has been designated hslv216.21. This clone has one nucleotide difference from framework region 1 (FR1)[1] through complementarity determining region 3 (CDR3) with a previously published gene segment, DPL13 (10). It is included in our analysis because it differs by three nucleotides in the 3′ flanking region from another gene hslv2132 which is identical to DPL13 and was obtained from the PCR library. Two genes were isolated from the 16.3 phage clone. Ψhslv216.376 a novel gene, is a pseudogene. The second gene hslv216.319 has only one nucleotide difference throughout the coding region from the previously reported gene DPL14 (10) and from hslv2011 obtained from the PCR library. It has not been included as a distinct gene in our analysis as the flanking region sequences are identical to those of hslv2011. The nucleotide sequence of the fourth gene hslv215.23, derived from phage clone 15.2, is identical to the previously reported DPL11 gene (10) from FR1 through CDR3 (Fig. 1) and is included to provide data on 5′ and 3′ flanking sequence. Hslv215.23, hslv216.21, and Ψhslv216.376 are aligned with other VλII germline genes (Fig. 1). In addition, the deduced amino acid sequences of the coding regions are presented (Fig. 2).

VλII genes obtained from a PCR library. Based on the restriction fragment length polymorphism analysis, the VλII family has been estimated to contain from 10 to 15 members (6, 11). Accordingly, to obtain additional germline genes from SLE patient K.S., a second library was generated by PCR amplification of VλII genes from total genomic DNA. 23 VλII clones were sequenced in their entirety, yielding eight distinct genes of which five were novel. Hslv2046, hslv2113, hslv2007, Ψhslv2120, and hslv2031 are reported in Figs. 1 and 2. Three genes identified from the PCR library were identical from FR1 through CDR3 to previously reported genes, hslv2066 to DPL10 (10), hslv2132 to DPL13, and hslv2011 to DPL14. 7 of the 23 were identical to other clones, six differed by only one nucleotide from either a genomic or PCR-derived clone and were arbitrarily deemed to represent polymerase errors. Two additional genes, hslv2018 and hslv2110, may represent chimeric molecules. They are included in Figs. 1 and 2 as our methodology appears not to generate such sequences, but they are not included in the subsequent analysis, because it is impossible to prove, at this time, that they are not artifacts. Thus Figs. 1 and 2 show a total of 13 genes obtained from the two libraries, aligned with other previously published VλII genes.

Sequence analysis. The VλII germline genes displayed in Fig. 1 show insertions, deletions, and conserved variable positions within the regions sequenced. Genes were considered different if they varied by two or more base pairs. Single base pair differences were arbitrarily assumed to be PCR artifacts. Table I shows the degree of homology among the VλII germline genes. The sequence of hslv216.21 differs from two other genes, hslv2007 and hslv2132, by a single base pair from the 5′ intron through CDR3, but potentially by three base pairs in the 3′ primer region. We cannot be certain about these differences as they occur within the primer used in the PCR amplification of hslv2007 and hslv2132. In addition, 89 base pairs of hslv216.21, from the leader region through the intron, were not present in the genomic clone. We are, therefore, unable to determine if it is indeed identical to either hslv2007 or hslv2132 or whether it has additional base changes that would result in its classification as a separate gene.

To understand the potential for somatic diversification of light chains encoded by members of this gene family, we analyzed the random replacement to silent mutation (R/S) ratio of each VλII germline gene. While a random R/S ratio calculated for the 61 triplet codons is 2.9 (14), the random R/S ratio for any particular gene or region of a gene may differ from this, reflecting the actual codon usage of the gene. The analysis of the random R/S ratio for each gene or segment of that gene, therefore, reveals the probability of obtaining an altered amino acid sequence, if mutations occur randomly with respect to location and base substitution. The R/S ratio of some VλII genes is higher than 2.9 in CDR1 and CDR3, implying a codon usage that favors mutability of these regions of the protein (Table II). While most members of the VλII family have the highest R/S ratio in FR2, the expressed antibody sequences do not reveal a high R/S ratio in FR2 suggesting there may be a selective pressure to conserve the amino acid sequence in this region.

Since we are interested in identifying VλII germline genes used in generating the expressed antibody repertoire and the 8.12 idiotype, we compared the VλII of germline genes to previously published nucleotide sequences of expressed VλII light chains. Four germline gene segments appear to encode all currently reported VλII encoded antibodies (Table III and Fig. 3). The hslv215.23/DPL11 gene shows 95–99% homology to the nucleotide sequences encoding 12 expressed light chains of which six are 8.12^+ (Table III). In addition, genes VλII.1/DPL10, hslv2066, hslv2046, and DPL12 also encode expressed VλII light chain genes. All these genes can encode 8.12^+ antibodies.

A characteristic of 8.12^+ antibodies that emerged from serum studies was their cationicity (3). The isoelectric point of

1. *Abbreviations used in this paper:* CDR, complementarity determining region; FR, framework region; R/S, random replacement to silent mutation ratio.

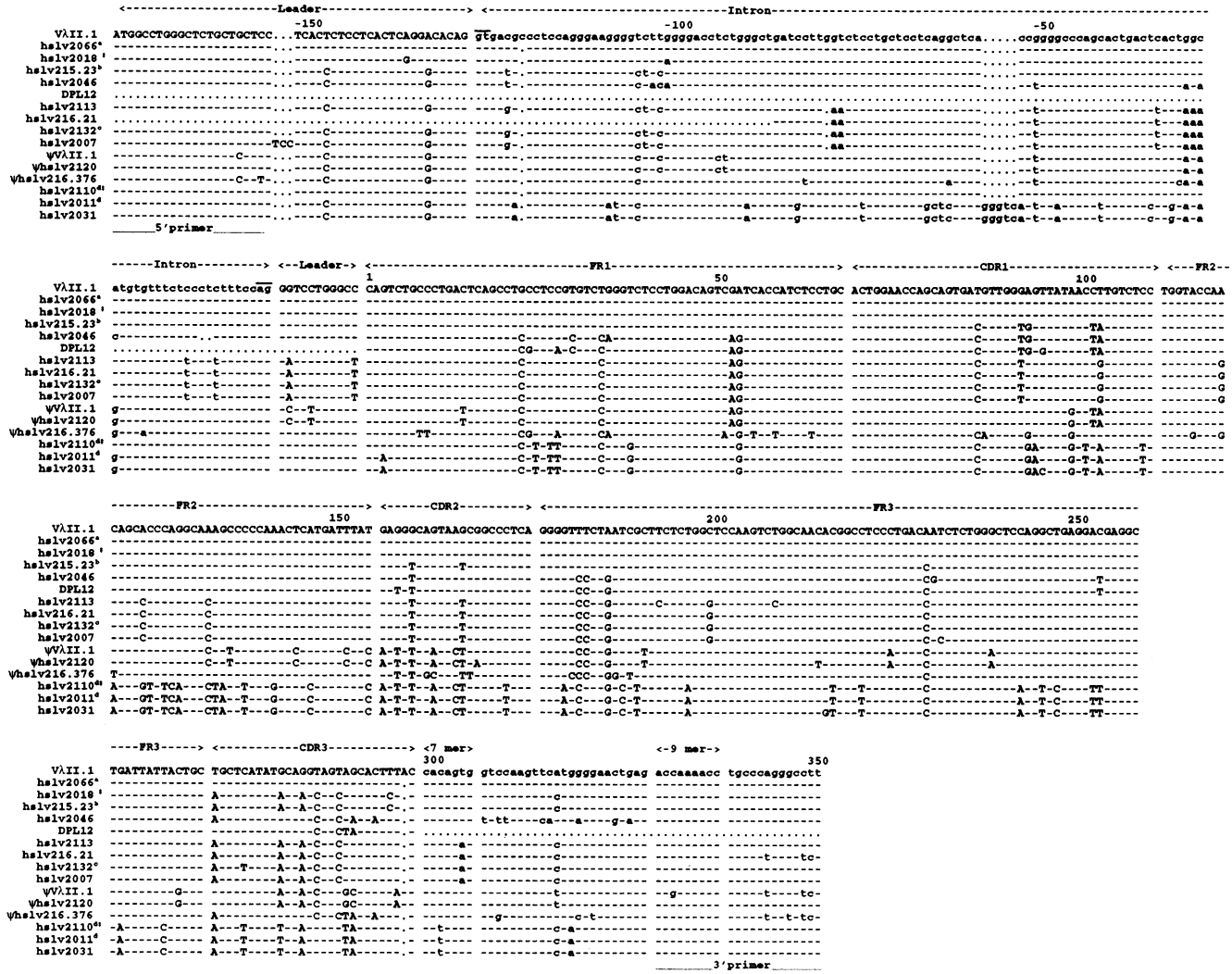

Figure 1. Nucleotide sequences of VλII germline genes. The sequences are aligned by homology to the VλII.1 germline gene. The primers used to obtain the PCR products are identified. Genes hslv216.21 and Ψhslv216.376 reflect genomic sequences through these regions, for all other genes the sequences reflect the primers. The sequences of VλII.1, DPL12, and ΨVλII.1 genes were taken from previous studies (9, 10 and 11, respectively). Dashes indicate sequence identity with the consensus sequence, VλII.1, only differences are shown. Dots indicate where gaps have been introduced to maximize alignment. The nucleotide sequences of the exons are shown with capital letters and those in the introns with lowercase letters. The 3′ heptamer and nonamer of RSS are shown. The FRs and CDRs are defined according to Kabat et al. (12). The RNA splice site in the intron area is show as a bar above. The sequences from genes [a–d] were identical from FR1 through CDR3 to the DPL10, DPL11, DPL13 and DPL14 genes, respectively (10). [‡]A chimeric sequence included in this figure and Fig. 2. The sequences are deposited in full in the EMBL/GenBank data libraries (accession numbers L27687-697, L27822, L27823).

the amino acid sequence encoded by each member of the VλII gene family was determined by computer analysis and there is no skewing of this family toward cationicity. Furthermore, even those VλII genes known to encode 8.12^+ antibodies do not display cationic isoelectric points (Table III).

VλII pseudogenes. The sequence of the Ψhslv2120 germline gene was found to be 99% homologous to a previously reported pseudogene, ΨVλII.1 (11) (Table I) and may, therefore, represent an allelic form of ΨVλII.1. Translation of the coding region of both genes shows an in-frame stop codon which renders them pseudogenes (Fig. 2). The gene Ψhslv216.376 also represents a pseudogene as translation of the coding region reveals stop codon in FRs.

VλII subgroups. Examination at the protein level of the expressed VλII segments and VλII germline genes reveals the key amino acids that define the VλII subgroups according to Chuchana et al. (Fig. 3) (13). Fig. 3 shows the comparison of the translated VλII germline genes to expressed VλII genes. All sequences can be ascribed to either the Con-IIa or Con-IIb subgroup (13), which are defined by particular amino acids in FRs 1 and 3 (Table IV). The germline genes hslv2031 and hslv2011/DPL14 while most like sequences in the Con-IIb subgroup, are the least homologous (78%) to other VλII genes and may represent a third subgroup in the VλII family. Since none of the expressed genes appears to derive from either of these two genes, they may represent pseudogenes (Fig. 3).

Discussion

We have analyzed the germline sequences of members of the VλII gene family derived from a single SLE patient. Altogether

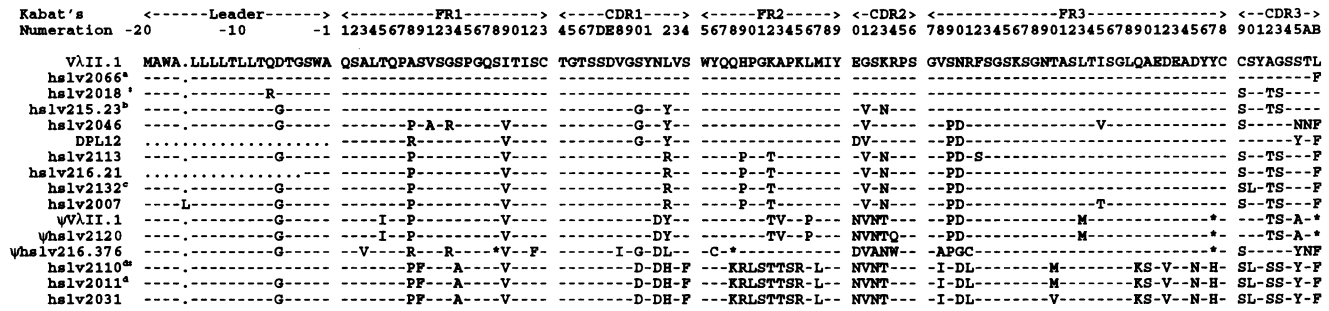

Figure 2. Deduced amino acid sequences of VλII germline genes. The sequences are aligned by homology to the VλII.1 germline gene (9). Dashes represent amino acid identity with VλII.1 and only differences are shown. Dots indicate where gaps have been introduced to maximize homology. ΨVλII.1, DPL12, and [a–d] nucleotide sequences are shown in Fig. 1 (11, 10). The leader region, and conventionally defined FRs and CDRs (12) are indicated. * is a stop codon. Codons are numbered according to the method of Kabat et al. (12), except for CDR1 which is according to Chuchana et al. (13). ‡ is a chimeric sequence.

we obtained 11 distinct sequences (excluding the two potentially chimeric sequences). Four of them are identical from FR1 through CDR3 to previously reported genes (10) and seven are novel. Three sequences were obtained from genomic clones, the other eight sequences were obtained from a library of VλII gene segments derived by PCR amplification of genomic DNA.

It is not possible without extensive mapping data to determine which sequences represent independent genes and which represent allelic forms of the same gene. Hslv2011/DPL14 and hslv2031 and ΨVλII.1 and Ψhslv2120 represent pairs of genes that differ by more than two nucleotides but are, nevertheless, sufficiently homologous that they may represent alleles. Hslv2132/DPL13 is highly homologous to three genes hslv216.21, hslv2007, and hslv2113. Since all were derived from a single individual, these four genes must represent at least two distinct gene loci. Analysis of the expressed VλII proteins suggests that other allelic forms may also exist. As displayed in Fig. 3, several antibodies derived from different individuals all possess an aspartic acid as the first amino acid in CDR2, while the most homologous germline gene yet reported encodes a glutamic acid. This difference reflects a single base change in the codon and thus may identify a second allele of the hslv215.23/DPL11 germline gene. Alternatively, it might represent a hot spot for somatic mutation. It is not clear whether alleles such as these that differ by only one or two amino acids could significantly alter the expressed antibody repertoire. Single amino acid differences in both immunoglobulin molecules and T cell receptors can affect cell receptor specificity (37, 38). While allelic differences may be critical in determining the T cell receptor repertoire, somatic mutation of immunoglobulin genes permits highly homologous alleles to acquire identical amino acid sequences. Genes differing by only a few base pairs, therefore, may not significantly alter the B cell repertoire.

A cluster analysis of the VλII genes, based on the percentage of homology from leader region to the nonamer, shows that hslv2011/DPL14 and hslv2031 are the most divergent VλII genes (Fig. 1 and Table I). These genes may represent a new subgroup of VλII. More probably, they are pseudogenes. Hslv216.319 (identical to hslv2011) is located adjacent to a known pseudogene Ψhslv216.376. There is a previous report

Table I. Percentage of Homology from FR1 to CDR3 Region among the VλII Germline Genes

	hslv2031	hslv2011/ DPL14	ψhslv2120	ψVII.1	ψhslv216.376	hslv2066/ DPL10	VλII.1	hslv215.23/ DPL11	hslv2007	hslv216.21	hslv2132/ DPL13	hslv2113	DPL12	hslv2046
hslv2031	100	99	80	81	78	80	80	81	81	82	82	81	82	81
hslv2011/DPL14		100	81	82	79	81	80	82	82	82	83	82	83	82
ψhslv2120			100	99	84	88	88	88	90	90	90	89	89	88
ψVλII.1				100	85	88	89	89	90	91	90	90	90	89
ψhslv216.376					100	87	87	88	89	89	89	88	91	90
hslv2066/DPL10						100	> 99	95	92	93	92	92	92	92
VλII.1							100	95	92	92	92	91	92	92
hslv215.23/DPL11								100	95	95	95	94	94	94
hslv2007									100	> 99	99	99	94	94
hslv216.21										100	> 99	99	94	95
hslv2132/DL13											100	99	94	94
hslv2113												100	93	94
DPL12													100	97
hslv2046														100

The actual nucleotide sequences of these genes are presented in Fig. 1. Homologies are reported as the percentage of identical nucleotides between two sequences, from FR1 through CDR3 (12).

Table II. Random Replacement (R) to Silent (S) Mutation Ratio of VλII Germline Genes

	FR1	CDR1	FR2	CDR2	FR3	CDR3
VλII.1	2.2	2.8	4.2	2.7	2.8	3.1
hslv2066/DPL10	2.2	2.8	4.2	2.7	2.8	3.5
hslv215.23/DPL11	2.2	2.9	4.2	2.7	2.8	3.5
hslv2046	2.2	2.9	4.2	2.7	2.7	3.8
DPL12	2.2	2.9	4.2	2.7	2.7	3.3
hslv2113	2.1	2.9	3.5	2.7	2.6	4.1
hslv216.21	2.1	2.9	3.5	2.7	2.8	4.1
hslv2132/DPL13	2.1	2.9	3.5	2.7	2.8	4.5
hslv2007	2.1	2.9	3.5	2.7	2.7	4.1
hslv2011/DPL14	2.3	3.9	2.9	2.4	2.8	4.2
hslv2031	2.3	4.3	2.9	2.4	2.6	4.2

To calculate the random R/S ratio for each region, each nucleotide within a codon is converted to each of the remaining three nucleotides, and it is determined whether this substitution results in a change in amino acid (R) or not (S). This analysis determines how likely it is that random mutation will result in an altered protein product. The sequences used in this analysis appear in Fig. 1. Pseudogenes are not included in the analysis.

of two pseudogenes being located adjacent to each other (39). Furthermore, the deduced amino acid sequences of these genes do not show more than 78% homology to any expressed protein. We would speculate that there are abnormalities in flanking regions that prevent effective transcription or rearrangement. The 3′ heptamer has a single base change that may affect rearrangement. While a heptamer with this sequence has not been previously described, single base substitutions of the heptamer have been reported to decrease the efficiency of V(D)J rearrangement (40). In addition, base substitutions occur in the heptamer region of S107 V13 gene of autoimmune mice (S. Behar and M.D. Scharff, unpublished data). This gene is rarely used in the expressed B cell repertoire. If hslv2011/DPL14 and hslv2031 are in fact pseudogenes, then five of 14 sequenced genes in the VλII family are pseudogenes.

It is apparent that many of the VλII germline genes from an SLE patient are identical to those of a nonautoimmune individual (38 and this report). These data suggest that an autoimmune individual may have the same gene repertoire as a normal individual, and that the V gene segments of nonautoimmune individuals can encode autoantibodies. The analysis of all VλII encoded antibodies, those with autospecificity and those binding foreign antigen, demonstrates that they are encoded by the same germline genes (Table III). Given the relatively high percent of pseudogenes in this family, one might have anticipated some diversification through gene conversion events, but our analysis of expressed VλII genes suggests that somatic point mutation is the major mechanism for somatic diversification of VλII genes.

The 8.12 idiotype, which is encoded exclusively by VλII genes (6), is present on serum immunoglobulin from normal and SLE individuals, but its expression is elevated in SLE patients (3). Our earlier findings suggested that the 8.12 idiotype is encoded in the germline gene sequence and that several germline genes can encode it (6). The present analysis shows that hslv215.23/DPL11, VλII.1, hslv2066/DPL10, hslv2046, and DPL12 can all encode 8.12^+ antibodies.

All three VλII encoded light chains that form anti-DNA antibodies are encoded by the hslv215.23/DPL11 germline gene

Table III. Replacement to Silent Mutation Ratios of VλII Encoded Antibodies

Antibody*	Specificity	8.12‡ Idiotype	pI§	Isotype	Germline gene	Homology¶	R/S (% R) FRs	R/S (% R) CDRs
PV11	not known	+	5.69	M	DPL11/hslv215.23	99	0:1 (0.0%)	2:0 (6.8%)
DSC	not known	+	7.17	M	DPL11/hslv215.23	99	0:0 (0.0%)	3:0 (10.0%)
DP2C	anti-red cell	ND	5.73	M	DPL11/hslv215.23	99	1:0 (1.4%)	2:0 (6.7%)
RT2B	anti-red cell	ND	5.70	M	DPL11/hslv215.23	99	1:1 (1.4%)	1:1 (3.3%)
JB21	Anti-Hib PS	ND	4.82	G	DPL11/hslv215.23	97	1:2 (1.4%)	8:1 (27.0%)
AS17	not known	−	4.51	G	DPL11/hslv215.23	96	1:1 (1.4%)	6:0 (20.0%)
KS3	anti-DNA	+	4.50	G	DPL11/hslv215.23	96	4:3 (5.8%)	5:0 (17.2%)
Ab2 383	anti-Id	ND	4.51	M	DPL11/hslv215.23	97	0:2 (0.0%)	6:0 (20.0%)
SD6	anti-DNA	+	6.49	G	DPL11/hslv215.23	95	3:2 (4.3%)	5:1 (17.2%)
33.H11	anti-DNA	ND	8.03	G	DPL11/hslv215.23	95	1:0 (1.4%)	4:0 (13.8%)
NGD9	not known	+	4.09	A	DPL11/hslv215.23	95	5:2 (7.2%)	3:2 (10.3%)
pMHL-1	not known	+	7.34	M	DPL11/hslv215.23	95	2:2 (2.9%)	6:4 (20.7%)
H210	anti-hepatitis A	ND	7.09	G	VλII.1/DPL10/hslv2066	98	1:2 (1.4%)	4:0 (13.8%)
A30c	not known	+	7.09	M	VλII.1/DPL10/hslv2066	96	0:2 (0.0%)	9:0 (30.0%)
PV6	not known	+	5.75	?‖	hslv2046	> 99	1:0 (1.4%)	0:0 (0.0%)
WLR	not known	ND	4.94	M	hslv2046	97	3:0 (4.3%)	4:2 (13.8%)
KS6	not known	+	7.87	G	DPL12	97	2:0 (2.9%)	5:1 (17.2%)
AS7	not known	−	6.48	M	DPL12	97	2:1 (2.9%)	4:2 (12.9%)

* The references for the antibodies are reported in Fig. 3. ‡ Reactivity; +, positive; −, negative; *ND*, not done. § The predicted isoelectric point for the amino acid sequences of the variable regions, excluding J, were obtained using the Genetic Computer Group (GCG) Program (Wisconsin). ‖ The isotype is not known. ¶ Percent nucleotide identity to the germline VλII sequence.

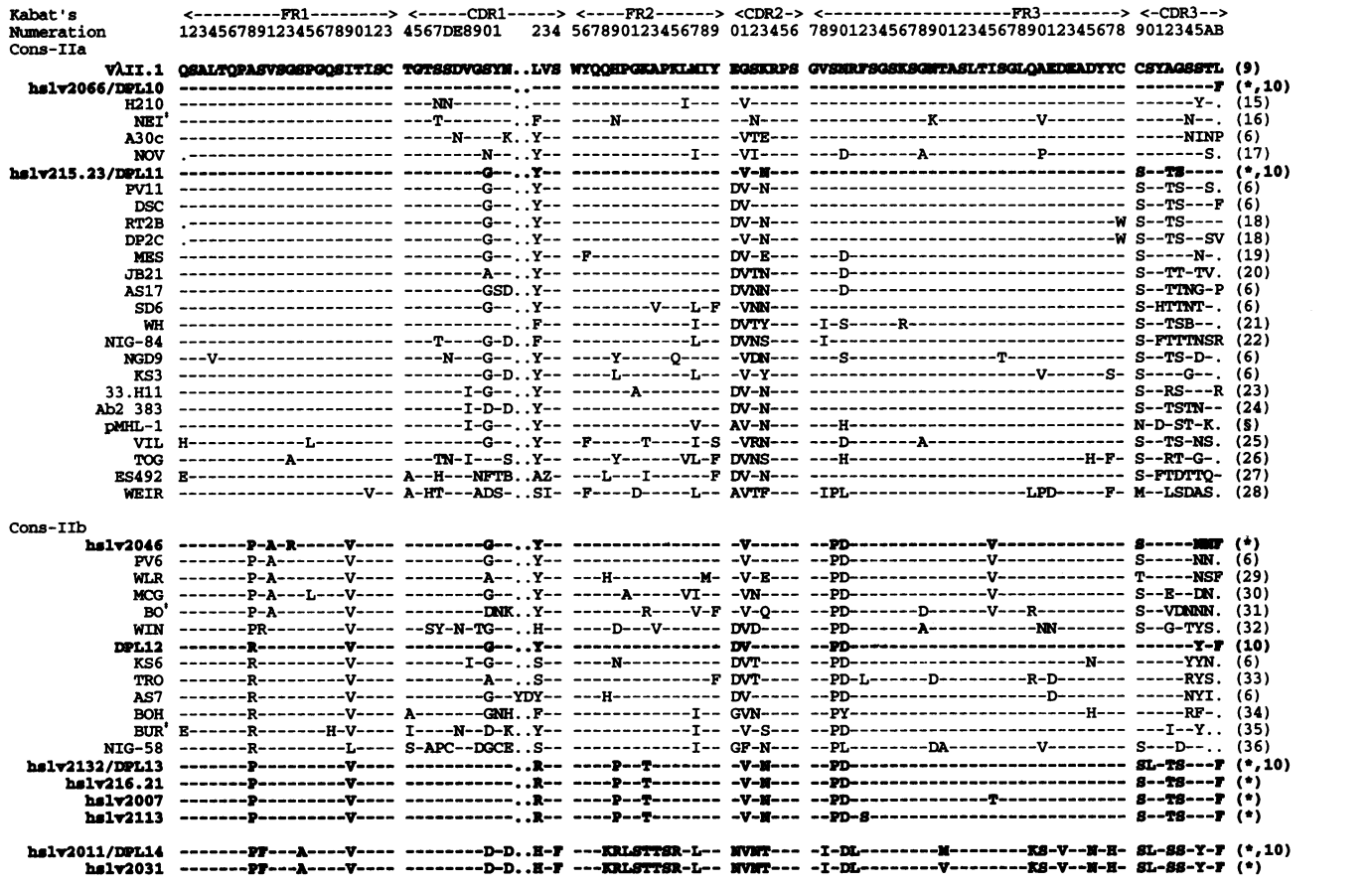

Figure 3. Amino acid sequences of reported germline and expressed VλII genes. Pseudogenes are excluded from this analysis. Germline sequences are in bold lettering. The other sequences are aligned under the germline gene to which they are most homologous. Dashes indicate amino acid identity to VλII.1 and dots are introduced to maximize homology. The numbers on the right represent the references from which the sequences were obtained. Subgroup and CDR1 numeration are derived from the classification of Chuchana et al. (13). * Deduced amino acid sequences of the germline genes isolated in this work. ‡ Discrepancies between Genbank and published sequences. § Genbank Sequence Search Nomura, N., K. Horigome, T. Nakatani, M. Terashima, and H. Noguchi. ‖ Genbank Sequence Search, Van der Heijdan, R. W. J., F. G. C. M. Uytdehaag, and A. D. M. E. Osterhaus.

(Table III) or a putative allele of this gene. This gene also encodes antibodies derived from nonautoimmune individuals with no known autospecificity (Fig. 3).

Serologic studies show the 8.12 idiotype is present almost exclusively on cationic antibodies (2) which have been shown to be preferentially deposited in glomeruli (41). Furthermore, the monoclonal 8.12$^+$ anti-DNA antibodies KS3 and SD6 are cationic (42). Nevertheless, VλII genes do not preferentially encode cationic protein sequences. Indeed, the light chain variable regions of KS3 and SD6 are anionic (42, Table III). Computer analysis of the amino acid sequence of the SD6 and KS3 heavy chain variable regions reveals a pI of 8.66 and 9.94, respectively (42), showing that the cationic charge of 8.12$^+$ antibodies may in some instances reflect pairing of an anionic light chain variable region with a cationic heavy chain variable region. A third VλII encoded DNA binding antibody 33.H11 has a very cationic light chain variable region, pI 8.03 (Table III). In this light chain, somatic mutation leads to the acquisition of positively charged amino acids in CDR3.

Table IV. Important Amino Acid Residues Differentiating the VλII Group, after Chuchana et al.'s Classification

Region	Position	Con-IIa	Con-IIb
FR1	8	A (26/26)	P (12/19)*
	19	I (26/26)	V (18/19)
FR3	59	S (25/26)	P (17/19)
	60	N (16/26)	D (17/19)

* Fractions represent the number of sequences containing the particular amino acid divided by the total number of sequences in the subgroup. The sequences used for this analysis are those presented in Fig. 3.

The analysis of the VλII genes of an SLE patient extends other studies that suggest that the defect that underlies autoimmunity is in the regulation of B cells. We have identified no major polymorphisms in VλII segments of an SLE patient. The same germline genes encode autoreactive antibodies in autoimmune individuals and anti-microbial antibodies from non autoimmune individuals. Somatic point mutation leads to diversification of expressed VλII gene sequences. While the database is small, there are no apparent differences in the frequency or location of mutations in the protective and the autoreactive response.

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