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Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype

CHRISTOPHE DEMAISON, PATRICIA CHASTAGNER, JACQUES THÈZE, AND MONCEF ZOUALI*

Unité d'Immunogénétique Cellulaire, Institut Pasteur, 75015 Paris, France

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ABSTRACT **Monoclonal anti-DNA antibodies bearing a lupus nephritis-associated idiotype were derived from five patients with systemic lupus erythematosus (SLE). Genes encoding their heavy (H)-chain variable (V_H) regions were cloned and sequenced. When compared with their closest V_H germ-line gene relatives, these sequences exhibit a number of silent (S) and replacement (R) substitutions. The ratios of R/S mutations were much higher in the complementarity-determining regions (CDRs) of the antibodies than in the framework regions. Molecular amplification of genomic V_H genes and Southern hybridization with somatic CDR2-specific oligonucleotide probes showed that the configuration of the V_H genes corresponding to V_H sequences in the nephritogenic antibodies is not present in the patient's own germ-line DNA, implying that the B-cell clones underwent somatic mutation *in vivo*. These findings, together with the characteristics of the diversity and junctional gene elements utilized to form the antibody, indicate that these autoantibodies have been driven through somatic selection processes reminiscent of those that govern antibody responses triggered by exogenous stimuli.**

The presence of autoantibodies to DNA is a hallmark of the systemic autoimmune disease called SLE (systemic lupus erythematosus). Since these autoantibodies play a major role in disease pathogenesis (1), determination of the genetic mechanisms that govern their production is currently the focus of considerable interest. The specificity of the antibody receptors expressed at the surface of B-cell subsets determines the dynamics of their growth and selection *in vivo*. Therefore, characterization of the variable (V) regions expressed by a B-cell clonotype can provide clues on the mechanisms of its selection (2). At the molecular level, maturation of antibody-producing cells is associated with a series of developmentally ordered molecular events that culminates in generation of a complete set of functional rearranged immunoglobulin heavy (H)- and light (L)-chain genes. After exposure to antigen, selected assembled *V* gene segments are driven to somatically mutate, and this, together with addition of imprecise joining (*J*) and *N* region nucleotide sequences, will give rise to high-affinity antibody binding domains (2, 3). In humans, analysis of the normal repertoire showed that polyreactive antibodies are produced predominantly by $CD5^+$ B cells that utilize preferentially germ-line gene-encoded segments (reviewed in refs. 4 and 5). To further our understanding of the molecular mechanisms that underlie the production of aggressive autoantibodies associated with SLE, we have characterized the genetic composition corresponding to the V regions of lupus antibodies that may be considered representative of nephritogenic anti-DNA autoantibodies. We have generated a panel of B-cell clones producing anti-DNA autoantibodies that bear the human pathogenic 0-81 idiotypic marker, an idiotype present in 72% of immune complexes and in 100% of renal eluates from patients with active SLE. In contrast to other lupus idiotypes, this marker is present in the circulation of most patients with active lupus nephritis but not in inactive SLE or healthy subjects (6). The features of the V_H sequences obtained, together with comparative PCR analysis, are indicative of somatic selection events that may have been responsible for their induction.

SUBJECTS, MATERIALS, AND METHODS

Generation of the Human Monoclonal Antibodies (mAbs). Mononuclear cells were prepared by Ficoll density gradient centrifugation of heparinized venous blood drawn from SLE patients who satisfied the revised American Rheumatism Association criteria for the classification of SLE. B cells were transformed with Epstein–Barr virus (EBV), and clones in positive wells were subcloned by limiting dilution. Immunoglobulin-containing culture supernatants were tested for binding to antigens by ELISA as described (7). Detection of 0-81 idiotype-positive antibodies was performed with a murine anti-idiotype mAb (6).

First-Strand cDNA Synthesis, dG-Tailing, and Anchored PCR. The first-strand cDNA was synthesized (20-μl final volume) at 42°C for 1 hr by using 10 μg of RNA, avian myeloblastosis virus (AMV) reverse transcriptase (Boehringer Mannheim), and an oligo(dT) primer. Second-strand synthesis and amplification were carried out via the PCR on one-eighth of the single-stranded cDNA reaction product in 50 μl (final volume) containing 200 μM of each dNTP, 50 mM KCl, 10 mM Tris·HCl (pH 8.3 at 37°C), 3 mM $MgCl_2$, 0.01% gelatin, 1 unit of *Taq* polymerase (Perkin-Elmer/Cetus), and 25 pmol of each primer. The two primers used were an anchor poly(C) primer and a μ H-chain constant (C) region gene (C_μ)-specific primer (positions 120–125) containing a *Not* I or *Hin*dIII restriction site at the 5′ end, respectively. Stringency of PCR amplification conditions was modified to yield an easily visible amplification product on ethidium bromide-stained agarose gels (8).

PCR Using V_H Family Leader Consensus Primers. Oligonucleotide primers were fashioned to enable PCR amplification of members of the different V_H gene families in conjunction with C_μ or C_γ primers. They were designed to hybridize to the leader sequences of the V_H region with a maximum mismatch of two nucleotides with a particular V_H gene family

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Abbreviations: SLE, systemic lupus erythematosus; CDR, complementarity-determining region; FR, framework region; H, heavy; L, light; R, replacement (mutation); S, silent (mutation); V_H, heavy chain variable region; EBV, Epstein–Barr virus; mAb, monoclonal antibody; V, variable, C, constant; J, joining; D, diversity.

*To whom reprint requests should be addressed.

of which the sequence was present in the GenBank nucleotide data bases.*

Cloning and Sequencing of the cDNA. The amplified DNA products were size-purified on an agarose gel by using DEAE membranes, digested by endonucleases, and ligated into pBluescript SK(+) (Stratagene), and the obtained recombinants were used to transform XL1-blue *Escherichia coli* cells (Stratagene). Recombinant plasmid DNA was alkali-denatured and sequenced on both strands by the dideoxy chain-termination procedure (8). The established V_H, H-chain gene diversity region (D_H), and (J_H) sequences were compared to available gene segment sequences from the GenBank data bank. Sequence alignments were verified manually.

PCR Amplification of Genomic V_H Genes and Southern Blot Hybridization Using Oligonucleotide Probes. Genomic DNA was isolated from B-cell clones and granulocytes as described (9). V_H genes were amplified by PCR under the following conditions: 30 μl final volume containing 100 ng of genomic DNA, 400 pM of dNTPs, 50 pmol of each primer, and 2.5 units of *Taq* polymerase at 94°C for 10 min followed by 30 cycles of 1.5 min at 94°C, 2 min at 72°C, and 5 min at 72°C. Twenty additional cycles were performed after addition of 10 μl of the same reaction mixture not containing genomic DNA. The sense and antisense primers were designed from the leader and framework region 3 (FR3) sequences of the V_H3 and V_H4 gene families, respectively. The PCR DNA product was size-fractionated on a 0.8% agarose gel and blotted onto Hybond N^+ filters (Amersham) by vacuum-transfer. Subsequently, filters were hybridized to V_H gene family cDNA probes as described (9). For hybridization with oligonucleotide probes, filters were prehybridized with 6× SSC (0.90 M NaCl/0.090 M sodium citrate, pH 7)/0.1% polyvinylpyrrolidone/0.1% Ficoll/0.1% bovine serum albumin/0.1% SDS/0.2 mg of tRNA per ml and were hybridized to the [γ-^{32}P]ATP-radiolabeled probe at a melting temperature (t_m) of −5°C. Filters were washed with 6× SSC/0.1% SDS at 20°C for 5 min and then at a final stringency of t_m = −5°C for 1 min.

RESULTS

V_H Gene-Family Utilization in Formation of Human Lupus mAbs. A series of B-cell clones were established by EBV transformation of the peripheral cells of five SLE patients. Following infection with EBV and culture for 2–4 weeks, plates with <20% of wells positive for cell growth were selected for study. This cut-off point provides for a statistical probability of monoclonality on the basis of Poisson distribution by limiting dilution protocols. mAbs produced by the clones were identified in a screening test for their capacity to bind native DNA and/or denatured DNA. After further subcloning by limiting dilution, we analyzed for binding to a panel of nine other antigens and for expression of the 0-81 nephritogenic idiotype (6). Six B-cell clones derived from five SLE patients were analyzed further; of them five expressed the 0-81 idiotype and produced antibodies that bound DNA but not nine other unrelated antigens (Table 1).

To determine the V_H gene family implicated in formation of the antibodies, a Northern blot analysis was performed by using purified RNA from each B-cell clone. The distribution of the V_H families in the clones (Table 1) did not significantly differ from that of the V_H distribution in EBV-transformed clones derived from healthy adults (5). Also, in comparison with the numbers of V_H genes with each family (4, 10–13), no significant deviation from a random utilization of the individual families was seen.

Nucleotide Sequence Analysis of the V_H Genes. The sequence of the V_H4 gene expressed by the T20-24 autoantibody exhibits 85% nucleotide sequence homology with that of the 58-P2 V_H gene that was cloned from human fetal liver (14). The differences between the two genes consist of four S differences and 11 R substitutions. At the amino acid level, the two sequences share 90% identity over the 105 positions. To rule out the possibility that the high load of variation seen in T20-24 V_H sequence was due to the experimental conditions used—EBV immortalization of the cells, culture conditions, PCR, cloning, and sequencing—we determined the nucleotide sequence of the V_H gene used by a B-cell clone designated T23-9, which was derived from a lupus patient, encodes an antibody with unknown binding specificity, and does not express the 0-81 idiotype. The V_H associated with the T23-9 antibody is related to V_H71-4 germ-line gene (10) but with four substitutions, only one of which results in an amino acid replacement. The slight departure from the germ line of this V_H gene could be due to either the usage of a different V_H71-4 allele or somatic mutations.

Four antibody-producing clones used V_H3 genes (Table 1). The T20-11 antibody utilized a V_H gene that is 94% identical to the M72 V_H gene isolated from fetal liver (15). The sequences of T21-9 and T24-3 V_H genes are identical to those of 56P1 and 60P2 V_H genes, also of fetal origin (14). The V_H gene of clone T34-1 (Fig. 1) is a derivative of the V_H26 germ-line gene (13).

Comparative Genomic PCR of the V_H Genes. If the substitutions observed in the V_H regions result from mutations that

*The heavy-chain sequences reported in this paper have been deposited in the GenBank data base (accession numbers X67906, X67907, X67908, X67909, X67910, and X67943 for T23-9, T24-3, T21-9, T34-1, T20-24, and T20-11, respectively).

Table 1. Immunochemical characteristics and *V* gene usage in the lupus-derived monoclonal antibody panel

Clonotype	Patient	Isotype	Idiotype	Binding specificity	Gene family*	Related V_H	Nucleotide identity, %	Deduced amino acid identity,† %	D_H‡	J_H
T20-11	DUF	γ1	0-81	DNA	V_H3	M72	94	93	Q52/13P1§	J_H6
T20-24	DUF	μ	0-81	DNA	V_H4	58P2	85	90	NA¶	J_H4
T23-9	OLL	μ	Negative	Unknown	V_H4	71-4	98	99	DN1	J_H4
T21-9	MEH	μ	0-81	DNA	V_H3	56P1	90	90	2P1	J_H3
T24-3	GOX	γ1	0-81	DNA	V_H3	60P2	88	82	15P1	J_H3
T34-1	GIR	μ	0-81	DNA	V_H3	V_H26	95	93	NA¶	J_H4

Expression of the nephritogenic 0-81 idiotype was determined by using the D1E2 murine mAb specific for the nominal 0-81 human idiotype (6). Binding to DNA was tested by ELISA with positive and negative antibody controls obtained from the AF-CDC ANA Reference Laboratory (Centers for Disease Control, Atlanta, GA). Antibodies of this panel did not bind to phosphorylcholine, ovalbumin, tetanus toxoid, tobacco mosaic virus protein, streptococcal A carbohydrate, thyroglobulin, actin, myoglobulin, and tubulin.

*Classification of V_H gene families is according to Lee *et al.* (10) and Berman *et al.* (11).

†Amino acid sequence homologies of deduced V_H gene products were determined for residues through position 94.

‡Designation of D_H gene segments is tentative and is based on homologies of >55% with germ-line D_H genes.

§Indicates a D_H gene used in an inverted orientation.

¶NA, not assignable.

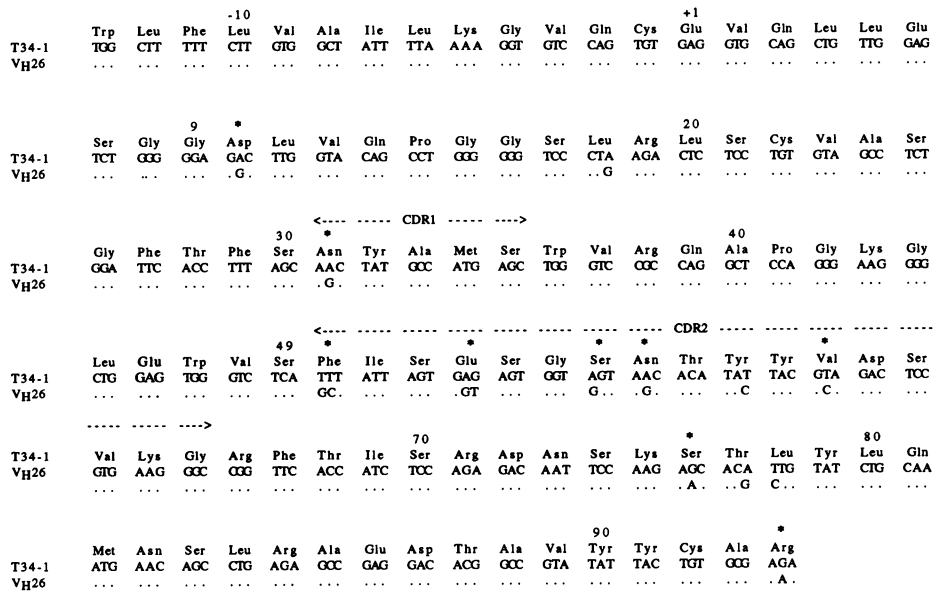

FIG. 1. Nucleotide sequence of the V_H gene and deduced amino acid sequence expressed by the SLE-derived T34-1 clone producing the T34-1 nephritogenic antibody. Asterisks denote replacement (R) mutations. The first and second complementarity-determining regions (CDRs) are overlined. Numbering is according to Kabat *et al.* (12). The sequence of the V_H26 gene was determined by Matthyssens and Rabbits (13).

occurred in the process of maturation of the B-cell clones, the somatic sequences must be absent in the patient's own germ-line DNA. To test this assumption, one V_H3 gene and one V_H4 gene that exhibited the highest divergence from their closest V_H gene relatives were selected for further study. T20-24 clone has a V_H gene related to 58P2 V_H4 gene and produces an IgM anti-DNA, 0-81 idiotype-positive antibody; T24-3 has a V_H gene related to 60P2 V_H3 gene and produces an IgG anti-DNA, 0-81 idiotype-positive antibody (Table 1). To prove that these two genes are the somatically mutated versions of the corresponding germ-line V_H genes, we performed the following experiments. Using sense and antisense primers corresponding to the leader and the FR3 regions of the V_H sequences, we PCR-amplified the V_H genes from genomic DNAs isolated from these two somatic B cells and from granulocyte preparations obtained from the two SLE patients whose cells were used to generate these B-cell clones (Fig. 2). The two antisense primers used are complementary to consensus sequences of the FR3 regions of V_H3 and V_H4, respectively. After PCR, reactions performed with DNAs isolated from the B-cell clones contained a DNA fragment that hybridized specifically with oligonucleotide probes corresponding to the CDR2 of the V_H gene products of either T20-24 or T24-3 B-cell clones. In contrast, reactions performed using DNAs isolated from the two patients' own granulocytes did not generate DNA fragments that hybridized with the somatic CDR2-specific oligonucleotides (Fig. 2).

To control for the ability of the DNAs to serve as a template for PCR amplifications of V_H genes, we hybridized the Southern filters to cDNA probes corresponding to the V_H3 and V_H4 whole regions. The PCR products of both germ-line and somatic DNAs were able to anneal to the cDNA V_H probes (Fig. 2), implying that the negative hybridizations observed with the CDR2 probes reflect the absence of the somatic sequences in the germ-line DNA of the patients of origin.

Sequence Analysis of the CDR3 Regions. The utilization of the J_H and D_H gene segments was determined by a computer research with germ-line J_H and D_H segments. The J_H segments utilized revealed a strong preference for J_H4 and J_H3 elements (16). mAb-encoding clones T24-3, T23-9, T20-24, and T21-9 have J_H genes with a number of mutations. T20-11 clone contains a truncated J_H6 gene starting at nucleotide position +3 and deviating from the germ-line by one substitution. Similarly, T34-1 clone has a truncated J_H4 gene starting at nucleotide position +12 and diverges from the germ-line sequence by two substitutions, one of them being silent. The use of truncated J_H genes in clones T20-11 and T34-1, and possibly clone T20-24, most likely results from an exonuclease activity. The other sequence differences in the J_H segments may be the result of allelic polymorphisms or somatic mutations.

The D_H segments in the mAb-encoding clones were very heterogenous and could be accounted for only partially by previously reported germ-line D segments. The presence of a D_H segment was established by using a minimum match of six nucleotides in a stretch of seven base pairs with a previously described D_H element. Fig. 3 displays the sequences of the D_H segments in the clones along with germ-line D_H relatives. These elements are remarkable for several reasons. They are heterogenous in length, ranging from short sequences of only nine nucleotides to larger D_H segments of up to 35 nucleotides (T34-1). Some of them have, at least at one end, a clear predominance of C and G over A and T, suggesting that at least part of the sequence could have been generated through N-region addition by terminal transferase. In some cases, the largest portion of the D_H segment appears to derive from a known germ-line D_H gene (T23-9).

In another example, the D_H gene seems to be expressed in an inverted orientation (T20-11). In three cases the CDR3 was composed of short stretches of multiple D segments both in direct and inverted orientations (T34-1, T20-11, and T20-24), but the homology was poor and it is difficult to decide which nucleotide derives from N-region addition and which from a D_H segment, so that assignment to a particular D_H segment is not certain.

DISCUSSION

In performing this analysis, we wished to examine (*i*) the proportion of the available V_H gene repertoire utilized in the aggressive anti-DNA response and (*ii*) the molecular basis of expression of potentially pathogenic lupus autoantibodies. To minimize the effects that might be introduced by the individuality of different SLE patients, we derived B-cell clones from several patients. Four of the five mAb-producing clones generated from several subjects utilize four different members of the V_H3 family. It is unlikely that this bias reflects

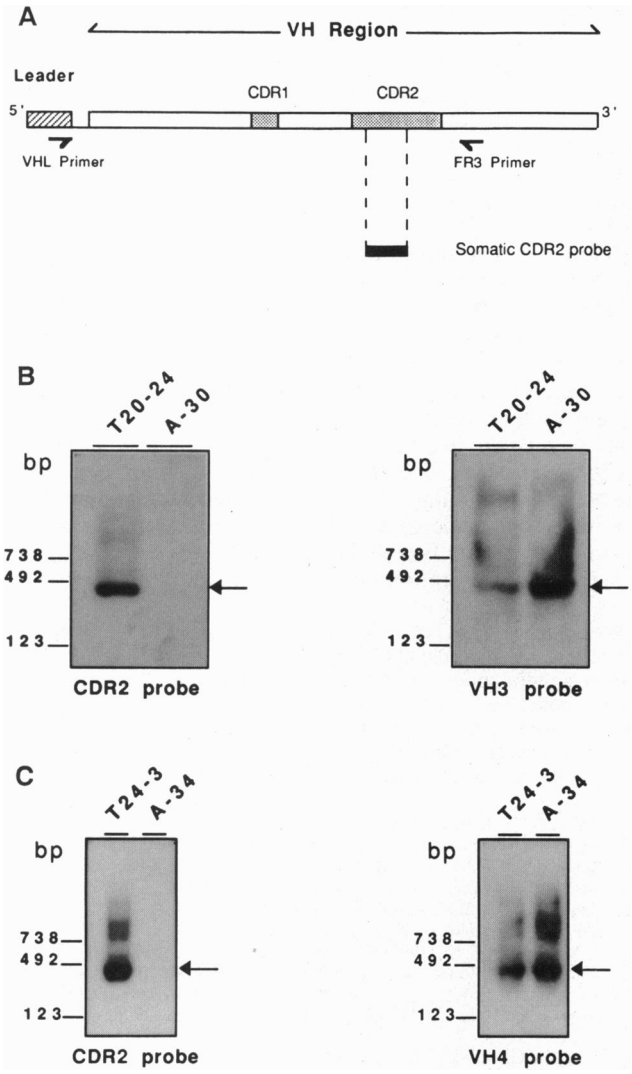

FIG. 2. Southern blot analysis of the V_H genes amplified from somatic DNAs of two B-cell clones (T20-24 and T24-3) and from the corresponding germ-line DNAs isolated from the granulocytes of the two patients of origin (A-30 and A-34). (*A*) Outline of the strategy used to show that the nucleotide substitutions observed in the somatic V_H genes are not present in the patient's own germ-line genes. Represented are the leader and the V_H regions. The CDR1 and CDR2 are also indicated. The sense (leader) and antisense (FR3) primers were used to PCR-amplify the V_H family genes. The two CDR2 probes used are homologous to the highly substituted portion of the CDR2 of the two somatic V_H genes expressed by the two B-cell clones. (*B*) One hundred nanograms of DNA was used to PCR-amplify V_H4 gene family members present in the genome of T20-24 B-cell clone and in the patient's own germ-line genome (A-30). The sense primer was homologous to the leader region of V_H4 genes (5′-GGGAATTCCTGGTGGCAGCTCCAGA-3′), and the antisense primer was from the FR3 region of 58P2 V_H gene (5′-GGTTCTTG-GACGTGTCTACGG-3′). The amplified DNAs were resolved by agarose gel electrophoresis and transferred onto a filter. The blot was hybridized to a CDR2 oligonucleotide probe (5′-GACAGCATTC-CCAGTAGG-3′) homologous to the CDR2 sequence of T20-24 V_H region. The same blot was stripped and rehybridized with a V_H4 gene-family-specific cDNA probe (see text for details). Depicted are the autoradiographs of the same filter hybridized sequentially with the radio-labeled oligonucleotide probe specific for the CDR2 probe and then with a cDNA V_H gene-family-specific probe. (*C*) A similar experiment was performed with T24-3 B-cell clone DNA and the patient's germ-line DNA (A-34). Here, the sense primer was homologous to the leader region of V_H3 genes (5′-GGGAATTCGAGTT-TGGGCTGAGCTGG-3′) and the antisense primer was from the FR3 of 60P2 V_H gene (5′-CGGCCGTGTCCTCGACTCTCAGGC-3′). The CDR2 probe (5′-GGTTCTCCCACTACTGCTC-3′) was homologous to the CDR2 sequence of T24-3 V_H region, and a V_H3 gene-family-specific cDNA probe was used. Exposures varied between 30 and 60 min.

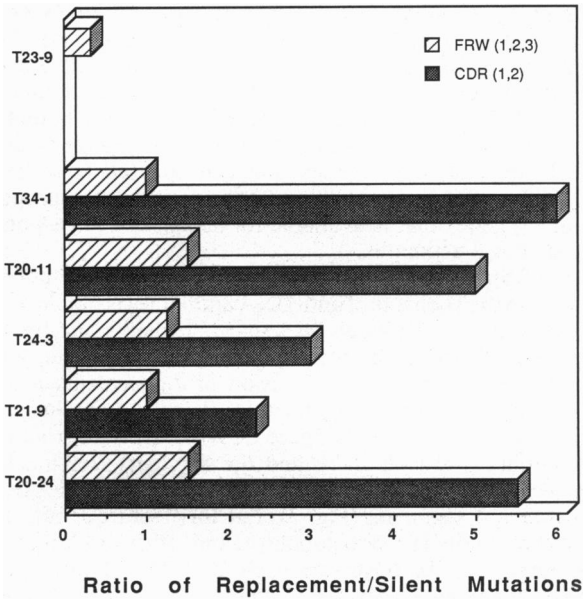

FIG. 3. Distribution of the mutations throughout the V_H regions of the antibodies. For each antibody, the ratio of R/S (silent) mutations was calculated for both the CDR1 and CDR2 (stippled bars) and FR1, FR2, and FR3 (hatched bars) of the V_H segments. T23-9 antibody was derived from a SLE patient, but its binding specificity is unknown, and it does not carry the 0-81 idiotype. The other antibodies may be considered representative of an aggressive subset of lupus anti-DNA autoantibodies.

a tropism of EBV for B-cell precursors expressing V_H3 genes. We and others have generated B-cell clones incorporating members of the various human V_H gene families (review in refs. 4 and 5). It is more likely that the biased use observed of V_H3 genes is related to their high genomic representation. The precise number of V_H3 germ-line elements has not been conclusively determined, but current estimates based on Southern blot analysis (11, 13), probing of the peripheral V_H repertoire by *in situ* hybridization (17), or by sequencing of V_H genes expressed by EBV clones (4, 5) or estimates obtained after PCR amplification of total peripheral cDNA (18) indicate that the V_H3 family dominates the human repertoire.

The mAbs we have analyzed are remarkable in several respects. They are complement-fixing antibodies, and their fine antigen specificity is typical of the human autoimmune response to DNA in SLE. They bound both denatured DNA and native DNA with a slight preference for the denatured DNA conformation but exhibited no cross-reactivity with a panel of auto- and exoantigens. They most likely recognize one strand of the DNA double helix and do not require the double-strand conformation of the B-DNA. They carried the 0-81 idiotypic marker, which is recurrently found in serum immunocomplexes and in renal lesions of nephritic patients (6). This idiotype is not present among antibodies of the normal human repertoire. These characteristics argue that the five antibodies we have analyzed are representative of a subset of aggressive autoantibodies that are involved in tissue lesions.

Overall, the results show that a substantial number of V_H genes are utilized in the expression of aggressive autoantibodies. Five different V_H genes were used to generate our antibody panel. Recently, other investigators have reported a total of 12 lupus anti-DNA antibodies that may also be considered representatives of aggressive autoantibodies (19–21). The corresponding V_H genes were also drawn from nine

different V_H elements belonging to the V_H3 and V_H4 gene families. It is currently thought that the human germ-line repertoire contains 100–200 nonallelic V_H genes with a least 40% pseudogenes (4, 5, 10–15). Based on these estimates, between 11% and 22% of the V_H repertoire has the potential to code for aggressive lupus autoantibodies. However, it is unlikely that all of the genes that can be used have been identified, and it is reasonable to anticipate that the proportion of V_H genes that is available for an aggressive response exceeds these estimates.

Apart from expression of different V_H segments, additional diversity in the H chains of anti-DNA antibodies is obtained by the expression of different D_H and J_H segments and by the generation of junctional diversity through deletion and *N*-region insertion. First, the expression of J_H elements in the antibody panel deserves comments. J_H3 and J_H4 are the most frequently expressed. This figure does not deviate from the preferential expression of J_H4 and J_H3 segments seen in adult peripheral B cells (22, 23). Some of our lupus antibodies have truncated J_H4 segments (Fig. 3), but this feature is not distinctive of these immunoglobulins, and other investigators have made a similar observation. Second, the CDR3 regions expressed are heterogenous in their size, their nucleotide sequence, and their predicted amino acid composition (16). In addition, their assignment to known D_H genes remains tentative. These characteristics most likely illustrate the complexity of human D_H genes. In contrast to murine D_H genes, they vary considerably in size. Their length can be as short as 3 nucleotides and may reach 60 nucleotides (4, 12). In addition, they exhibit a tremendous sequence heterogeneity, and when known D_H sequences are compared with each other, it is difficult to find significant homology between D_H genes. Furthermore, unconventional rearrangement mechanisms are observed in a high proportion of CDR3 sequences (22, 23). They involve D_H–D_H fusions, use of D_H-gene-containing irregular spacer signal (DiR) elements, and inversion of D_H segments. The origin of some of the D_H genes identified herein cannot be explained by these mechanisms. Therefore, it is plausible that a number of other germ-line D_H genes are yet to be isolated and/or additional rearrangement mechanisms are involved in generation of the corresponding CDR3.

The growth and selection of B cells *in vivo* are dependent on the specificity of the antibodies expressed at their surface. Therefore, molecular analysis of the antibodies expressed in various B-cell subsets can shed light on the mechanisms by which cells are selected. Somatic hypermutation is an important mechanism during the antibody response; it induces extensive changes in the structure and function of the antibodies. Hybridomas generated at late stages of the immune response correspond to *V* region genes with nonrandom distribution of somatic mutation. The ratio of mutations causing R to those that cause S mutations is often very high in the CDRs and low in the FRs (24). So too were the distributions of R/S ratios among the five 0-81 idiotype-positive nephritogenic antibodies. This pattern was not seen in the control antibody we have examined (Fig. 3). This result supports the view that selection was responsible for the mutations of the five antibodies. Inasmuch as all of the DNA-specific idiotype-positive antibodies we have analyzed are molecularly diversified, one may conclude that there is a correlation between monospecificity, idiotype expression, and somatic mutation.

To prove that the antibodies are indeed the product of somatic diversification, we used a comparative PCR approach to show that the somatic sequences expressed in the B-cell clones were absent in the germ-line DNA of the patient of origin. We would also like to emphasize that IgM anti-DNA antibodies bearing the pathogenic idiotype were purposely included in this study. Since class-switching is usually associated with somatic mutations, it was reasoned that finding variations in antibodies of the IgM isotype would lend further support to the conclusion that these molecules have undergone diversification. Although more commonly observed in the late primary response, there is evidence that mutations may occur as early as day 5 after primary immunization (25).

Our findings that somatic diversification is characteristic of human lupus anti-DNA antibodies agree with related observations (19–21). If, as the above data suggest and as has been found in lupus strains of mice (24), antibodies are generated by an antigen-driven mechanism, what is the nature of the driving force? The role of DNA or a cross-reactive antigen is suggested by observations in the murine system, where it was shown that, upon somatic mutation, antibodies may acquire a native DNA binding specificity (25, 26). Overall, the genetic composition of lupus nephritogenic autoantibodies suggests that they could not result from a mere polyclonal B-cell activation process. The high-load and distribution pattern of the somatic mutations is rather consistent with an immunoglobulin-receptor-dependent selection process. However, how DNA-specific B-cell clonotypes undergo selection, expansion, and maturation *in vivo* remains to be understood.

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