Somatic Mutations in the Variable Regions of a Human IgG Anti-double-stranded DNA Autoantibody Suggest a Role for Antigen in the Induction of Systemic Lupus Erythematosus

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Summary

The processes that govern the generation of pathogenic anti-DNA autoantibodies in human systemic lupus erythematosus (SLE) are largely unknown. Autoantibodies may arise as a consequence of polyclonal B cell activation and/or antigen-driven B cell activation and selection. The role of these processes in humoral autoimmunity may be studied by molecular genetic analysis of immunoglobulin (Ig) variable (V) regions of antibodies that are characteristic of SLE. We have analyzed the gene elements that encode a high affinity, IgG anti-double-stranded DNA autoantibody secreted by a monoclonal Epstein-Barr virus (EBV)-transformed cell line derived from a patient with active SLE. In addition, we have identified, cloned, and sequenced the germline counterparts of the VH and VL genes expressed in this autoantibody. The comparison of both sets of gene elements shows that the autoantibody VH and VL regions harbor numerous somatic mutations characteristic of an antigen-driven immune response. The light chain expressed in this autoantibody is a somatically mutated variant of the kv325 germline gene that is frequently associated with paraproteins having autoantibody activity and with Ig molecules produced by malignant B cells that express the CD5 antigen. Furthermore, the utilized DH segment has been repeatedly found in multireactive, low affinity IgM anti-DNA autoantibodies from SLE patients and healthy individuals. These results suggest that pathogenic IgG anti-DNA autoantibodies in human SLE may arise through antigen-driven selection of somatic mutations in the gene elements that frequently encode multireactive IgM autoantibodies.

Systemic lupus erythematosus is an autoimmune disease characterized by the production of antibodies that react with a variety of ubiquitous autoantigens including ribonucleoproteins, cytoskeleton proteins, and nucleic acids, such as single-stranded (ss)1 and double-stranded (ds) DNA (reviewed in reference 1). A direct role for IgG anti-DNA antibodies in pathogenesis has been established by the correlation of serum antibody levels with disease activity (2) and the demonstration of DNA/anti-DNA immune complex-depositions at sites of tissue damage (3). Although human anti-DNA antibodies have been extensively characterized by immunochemical, idiotypic, and structural analyses, (e.g., 4–7) the processes that lead to the production of these autoantibodies in SLE are largely unknown. Two, not mutually exclusive models have been proposed to explain their origin. The first model suggests that anti-DNA autoantibodies merely arise through antigen independent, polyclonal B cell activation (8, 9). This model denies a role for antigen in the autoantibody response and predicts the generation of a multilocal population of B cells with unmutated or randomly mutated Ig V regions. The second model proposes a role for (auto)antigen in the induction and maintenance of autoantibody production. This scenario predicts the generation of an oligoclonal population of B cells that express V regions with nonrandom patterns of somatic mutations. These patterns are presumably the result of positive selection of replacement mutations in parts of the V region that are in contact with antigen which leads to higher antibody affinity (10–12).

1Abbreviations used in this paper: ds, double stranded; ss, single stranded.
Therefore, the molecular analysis of the V regions that encode disease-associated anti-DNA autoantibodies will contribute to our understanding of the mechanisms involved in humoral autoimmune disease.

Recently, several groups have reported on the analysis of V genes expressed in human IgM anti-DNA autoantibodies derived from both patients and healthy individuals (13–18). Typically, these are low affinity, often broadly crossreactive antibodies that are encoded by strongly conserved, germline V regions. Whether these naturally occurring antibodies can give rise to high affinity, monospecific IgG anti-DNA autoantibodies characteristically present in the sera of patients with SLE is unknown.

Here, we present the binding characteristics, idiotype expression, and complete nucleotide sequences of the expressed VH and VL genes of a high affinity, monoreactive IgG anti-DNA autoantibody secreted by an EBV-transformed cell line derived from a patient with active SLE. In addition, we present the nucleotide sequences of the patients’ germline counterparts of these expressed VH and VL genes. Comparison of these sequences suggests that this IgG anti-DNA autoantibody-secreting B cell clone arose as a consequence of antigen-dependent stimulation and selection through its Ig receptor. The gene elements that encode this Ig receptor are frequently associated with IgM autoantibodies present in patients and healthy individuals.

Materials and Methods

Generation of Monoclonal IgG-Secreting EBV-Transformed B Cell Lines. Peripheral blood mononuclear cells from a patient with active SLE (Tou) were isolated and depleted of T cells using SRBC as described elsewhere (19). To increase the proportion of IgG-secreting cell lines, non-T cells were depleted of surface-IgM+ B cells by rosetting with anti-human IgM-coated ox red blood cells as described (20). The slgM-depleted non-T cells were incubated with the EBV-containing supernatant of the marmoset B95.8 cell line and cultured in 96-well plates under limiting dilution conditions (21). After 3–4 wk, clones from 96-well plates containing <25% growth were assayed for Ig secretion and expanded.

Assay for Ig Isotype. The isotype of secreted Ig was determined in direct binding ELISA using affinity-purified goat anti-human 
\( \mu \), \( \gamma \), \( \alpha \), and \( \kappa \) specific antisera (Tago Inc., Burlingame, CA) as described (16).

Assay for Antigen Binding Properties and Id Expression. Culture supernatants were assayed for anti-ssDNA and anti-dsDNA activity using a fluid phase binding assay (22). Preparation of photobiotinylated DNA and assay conditions were exactly as described (22) with one modification: microtiter plates were coated with 5 µg/ml goat anti-human IgG antibody (Tago Inc.) rather than goat anti-mouse antibody. Anti-dsDNA binding activity was also tested in an immunofluorescence assay employing Chlamydia psittaci I hemolysates as a substrate (23). Culture supernatants with anti-DNA binding activity were further analyzed for binding activity against a panel of 13 antigens using direct binding solid phase ELISA. The panel of antigens consisted of bovine heart cardiolipin, BSA, dinitrophenol-BSA, trinitrophenol-BSA, asarone-BSA, fluoresceine-BSA, rabbit IgG, human insulin, human thyroglobulin, hen egg lysozyme, tetanus toxoid, cytochrome C, and rabies virus. Controls, coating, and assay conditions were as described elsewhere (16).

IgG anti-DNA antibodies were assayed for L chain-associated Id expression in an ELISA system using the 17.109 and JG-B1 mAb (24, 25).

DNA and RNA Isolation. Total RNA was isolated from the EBV-transformed cell lines using RNAzol according to the manufacturer’s protocol (Cinna/Biotec, Friendswood, TX). Genomic DNA was isolated from 3.10^6 T cells, granulocytes, and EBV-transformed B cells as described (26).

First Strand cDNA Synthesis and PCR. For first strand cDNA synthesis, 9 µg of total RNA was annealed to 100 ng of a Cy or Cx-specific primer (TL 50 and TL 51, Table 1) and extended with AMV reverse transcriptase (Pharmacia, Uppsala, Sweden) using standard procedures (27). For PCR amplification, 1/10 of the cDNA first-strand reaction mixture or 50 ng of the isolated genomic DNA was supplemented with 500 ng of the appropriate 3' and 5' primers and amplified in a 100 µl reaction mixture using 3 U Taq DNA polymerase according to the manufacturers protocol (Promega Biotech, Madison, WI). The 5' primers used for these experiments (Table 1) were designed to anneal to the leader sequences of members of the human VH (TL 6, 15, 46, 47, 48) or VK (TL 40, 41, 43, 52) gene families based on published sequences (EMBL database, release 23). The 3' end primers were the Cy and Cx primers used in cDNA first strand synthesis or, for genomic DNA, primers that hybridize to 3' end genomic flanking regions of the VH4 or VKIIIb genes (based on 27–29). To facilitate subcloning, all primers contained an attached or internal restriction site (Table 1). PCR amplifications were carried out in a Bioexcellence Thermal Cycler and consisted of 35 cycles of 1.5 min of denaturation at 94°C, 1.5 min of primer annealing at 55°C, and 3 min of extension at 72°C. Finally, after 35 cycles, reaction mixtures were incubated at 72°C for 10 min to ensure full extension of all PCR products. First strand cDNA and PCR reactions were set up in duplicate in order to minimize errors introduced by AMV reverse transcriptase and/or Taq polymerase.

Cloning and Sequencing of PCR-Amplified Material. PCR-amplified material was separated by 3% agarose gel using affinity-purified goat anti-human \( \mu \), \( \gamma \), \( \alpha \), and \( \kappa \) specific antisera (Tago Inc., Burlingame, CA) as described (16). The 3' end primers were the Cy and Cx primers used in cDNA first strand synthesis or, for genomic DNA, primers that hybridize to 3' end genomic flanking regions of the VH4 or VKIIIb genes (based on 27–29). The Cy and Cx-specific primers were then used in cDNA first-strand synthesis and PCR amplification. The 3' end primers were the Cy and Cx-specific primers (Table 1) and extended with AMV reverse transcriptase (Pharmacia, Uppsala, Sweden) using standard procedures (27). After transformation of competent DH5α cells, colonies were transferred to nitrocellulose and screened with [32P]labeled Cy and Cu-specific probes (28, 29). Dideoxy sequencing was carried out using double stranded DNA and the T7 sequencing kit (Pharmacia). All inserts were sequenced from two directions and from multiple independent clones.

Results

Generation of an IgG Anti-DNA Secreting EBV-Transformed Cell Line. Monoclonal cell lines were generated by EBV-transformation of slgM-depleted non-T cells of SLE patient Tou under limiting dilution conditions. Supernatants of transformed cell lines were screened for the isotype of the secreted Ig in an ELISA. Subsequently, anti-DNA binding properties of IgG-producing cell lines were determined in a fluid phase assay using photobiotinylated ssDNA and dsDNA. The supernatant of one cell line (T14) out of 72 IgG-secreting cell lines tested displayed anti-ssDNA and anti-dsDNA binding activity (Fig. 1). Binding of T14 supernatant to dsDNA was confirmed in an immunofluorescent assay employing the hemoflagellate C. psittaci, which detects high affinity autoan-
The nucleotide sequences of the VH and VL regions of T14 (VH4.T14 and VKIIIb.T14 respectively) are shown in Figs. 2 and 3. Comparison of these sequences with sequences in the EMBL data-bank revealed that VH4.T14 is most homologous (95.8%) to the VH4.21 germline gene (Fig. 2; reference 30). Other germline VH4 genes displayed 91.1% or less sequence homology to VH4.T14 (Table 2; references 30, 31). The L chain expressed in T14 displays most homology to the kv325 germline gene segment (98.6%), a member of the VKIIIb subfamily (reference 32; Fig. 3). Other VKIII sequences displayed 96.7% or less sequence homology to VKIIIb.T14 (Table 2; reference 33).

The nucleotide sequence of a stretch of 90 nucleotides at the 5' end of the γ C region gene included in our PCR-amplified material revealed that autoantibody T14 is of the IgG3 subclass.

Cloning and Sequencing of Germline V Genes. We wished to determine if the V genes expressed in T14 indeed derived from the published germline VH4.21 and VKIIIb elements as suggested by the comparison with sequences in the EMBL databank. Such an analysis is necessary to determine whether nucleotide differences between expressed and published germline V genes do represent somatic mutations or, alternatively, reflect allelic heterogeneity or the existence of as yet unknown V gene elements. To that end, we used two approaches. First, we used the PCR to determine whether the 'mutated' VH4 CDR1 sequence of cell line T14 existed in the germline DNA of patient Tou (14). PCR reactions used a common 3' end primer that hybridized to framework 3 sequences of VH4 genes (TL90) and either a 5' end primerspecific for CDR1 of the germline VH4 gene (TL91) or a 5' primer specific for

Cloning and Sequencing of the Expressed V Regions. For cloning of the expressed VH and VL regions of T14, total cellular RNA was annealed to Cγ- or Ck-specific primers and extended with AMV reverse transcriptase. Subsequently, first strand cDNA was amplified in the PCR reaction using combinations of the Cγ or Ck primer and primers specific for the leader sequences of several human VH or VK gene families respectively (Table 1). For both H and L chain, a single primer combination (Cγ and VH4 for the H chain and Ck and VKIII for the L chain) resulted in the generation of PCR-amplified material of the expected size. The expression in T14 of genes belonging to the VH4 and VKIII gene families was confirmed in a Northern blotting experiment using the different VH and VK gene family-specific probes (not shown). The PCR-amplified material was cloned into Bluescript and multiple colonies containing the correct insert as detected by colony hybridization with VH4 and VKIII-specific probes were selected for dideoxy sequencing.

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Figure 2. Comparison of nucleotide sequences of the germline Tou-VH4.21 gene and the VH4.T14 gene expressed in cell line T14. Stripes indicate nucleotide identity. (*) replacement mutation. A: single nucleotide difference with the published VH4.21 sequence (reference 31). CDR: complementarity determining region. These sequence data are available from EMBL/GenBank/DDIR under accession numbers X56591 and X56592.
Table 1. Nucleotide Sequences of Primers Used in First Strand cDNA and PCR Reactions

| Primer no. | Specificity   | Sequence (5'→3')                      |
|-----------|--------------|---------------------------------------|
| TL50      | Cgamma       | ggaattaCAGGCAGCCAGGGCGCTGTGC          |
| TL50      | Ckappa       | ggaattaAACAGAGGCAGTTCCAGACTT          |
| PCR primers |             |                                       |
| TL46      | VH1 leader   | ggggaatcATGGGAGCTGGATTGAGGGG         |
| TL47      | VH3 leader   | ggggaatcGAATTGGGCTAGCTGGG            |
| TL48      | VH4 leader   | ggggaatcCTGTTGGGCACCTCCAGA           |
| TL15      | VH5 leader   | ACCGgaattTCGCCCTCCTCTTG              |
| TL6       | VH6 leader   | TGTagcctCTCATCTTCT                   |
| TL40      | VK1 leader   | ggggaatcATGGGACATGGGTTCCCC           |
| TL41      | VK2 leader   | ggggaatcATGGAGCTCCCTGTCAG            |
| TL52      | VK3 leader   | ggggaatcATGGAAGCCAGCCGCCA           |
| TL43      | VK4 leader   | ggggaatcATGGGTCAGCAGCCAG             |
| TL65      | VH4 3' flank | ggggtcgacGGGTCACAACACTCCCTCCCT            |
| TL75      | kv325        | ggggtcgacTAAACAATAAGGGTCTGGCA        |
| TL91      | VH4.21 CDR1  | TGTTGGGTCCTCATGTTACTA                |
| TL92      | VH4.T14 CDR1 | TGTTGGGTCCTCATGTTACTT               |
| TL90      | VH4.FR3      | ACAGTAATACAGCCGTCGTCGCC             |

Sequences of oligonucleotide primers used for first strand cDNA synthesis and PCR amplification of V genes. Capital letters refer to variable or constant region sequences. Restriction sites are underlined.

The CDR1 of the 'mutated' VH4 gene (TL 92), 'Germline' primer TL91 will also amplify the closely related V58 gene (31) and possibly additional, not as yet identified, VH4 genes with identical CDR1 regions. The PCR reaction was per-

Figure 3. Comparison of the nucleotide sequence of the germline Tou-kv325 gene segment and the VKIIIb gene segment expressed in the IgG anti-DNA autoantibody T14. The Tou-kv325 gene segment is 100% identical to the previously published kv325 consensus sequence (33). For explanation see legend to Fig. 2. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers X56593 and X56594.

Table 2. Comparison of VH4-T14 and VKIIIb-T14 with Published Germline VH4 and VKIII Genes

| Gene         | Percent sequence identity with VH4-T14 | Percent sequence identity with VKIIIb-T14 |
|--------------|----------------------------------------|------------------------------------------|
| VH4.21       | 94.8                                   | 98.6                                     |
| V71-4        | 89.0                                   | 96.7                                     |
| V11          | 87.1                                   | 93.0                                     |
| V12G-1       | 85.7                                   | 92.3                                     |
| V58          | 91.1                                   | 91.3                                     |
| V2-1         | 86.5                                   |                                           |
| V79          | 87.4                                   |                                           |
| V71-2        | 87.9                                   |                                           |
| 2-9II        | 86.7                                   |                                           |
| Tou17s       | 85.9                                   |                                           |
| Tou10s       | 87.6                                   |                                           |
| Tou-VH4.21s  | 95.1                                   | 98.6                                     |

Percentage of nucleotide identity between the VH4 and VKIIIb genes expressed in T14 and germline VH4 and VKIIIb sequences.

* Germline VH4 sequences from references 30 and 31.
† Germline VKIIIb sequences from reference 33.
§ Germline VH4 and VKIIIb sequences from patient Tou.
Table 3. Pattern and Extent of Somatic Mutations in the V Regions of Cell Line T14

| CDR regions | FR. regions |
|-------------|-------------|
|             | Silent      | Replacement | Silent | FR. |
| VH          | 2           | 4 Tyr → His | 5      | 2 Ser → Arg |
|             |             | Tyr → Phe  |        | Val → Ala  |
|             |             | Glu → Asp  |        |            |
|             |             | Asn → Ser  |        |            |
| VL          | 0           | 3 Ser → Asn | 1      | 1 Gly → Ala |
|             |             | Ser → Arg  |        |            |
|             |             | Ser → Thr  |        |            |

The three-letter amino acid code is used. CDR, complementarity determining region. FR, framework region.

The data are presented in Table 3, which shows the pattern and extent of somatic mutations in the V regions of cell line T14 formed using three sources of genomic DNA: (a) DNA from cell line T14, (b) DNA from peripheral blood T cells from patient Tou and as a control, (c) DNA from the granulocytes of a healthy, unrelated person. In all experiments, the identity of fragments generated in the PCR was confirmed in a Southern blotting experiment using a \[^{32}P\]-labeled VH4-specific probe. The results of these experiments are shown in Fig. 4. A combination of the germline CDR1 primer (TL90) and TL91 generated a VH4-hybridizing band of the expected size (205 bp) in all three DNA samples, indicating the presence of VH4 genes (such as VH4.21 and V58) containing this CDR1 region. In contrast, a combination of the 'mutated' CDR1 primer (TL92) and TL91 generated a VH4-hybridizing band in the DNA sample from cell line T14 only. Together, these data demonstrate that the mutated CDR1 sequence exists in the rearranged DNA of the T14 cell line but not in T cells from the same donor or in granulocytes from a control donor. Therefore the nucleotide sequence substitutions in the CDR1 region of cell line T14 result from

Figure 4. PCR analysis of somatic mutations in the expressed VH4 gene in cell line T14. (a) Diagramatic representation of the prototypic germline VH4 gene (VH4.21) and the expressed VH4 gene segment (T14) with locations and directions of PCR primers TL91, TL92 (5' end primers), and TL90 (3' end primer). (b) Ethidium bromide-stained gel showing amplified products. (c) Southern blots showing specific amplified products after probing with \[^{32}P\]-labeled VH4 probe. Genomic DNA was isolated from the cell line T14, from T cells of patient Tou, and from granulocytes from a control donor. Lanes 1, 5, and 9, genomic DNA from T cell of patient Tou; lane 2, 6, and 10, DNA from cell line T14; lane 3, 7, and 11, DNA from granulocytes of a control donor; lane 4, 8, and 12, negative controls (no DNA added); lane 1-4, primers TL91 and TL90; lane 5-8, primers TL92 and TL90; lane 9-12, primers TL65 and TL48.
demonstrated the absence of this mutated sequence in the germline DNA of patient Tou. Again these experiments displayed a higher degree of homology with the sequence of genes, including two new members of the VH4 gene family, derived from this allele. Furthermore, none of the other 24 VH4 genes present in the expressed VH4 gene in cell line T14, rendering it highly likely that the expressed VH4 gene was derived from the patient Tou's own germline DNA. Single-letter amino acid code is used. Dashes indicate identity with the germline sequence.

somatic mutations. The same approach was used to establish the absence of the mutated VKIIIb CDR1 sequence in the germline DNA of patient Tou. Again these experiments demonstrated the absence of this mutated sequence in the germline of the SLE patient Tou (not shown).

In the second approach, we cloned and sequenced 25 VH4 genes from the genomic DNA of patient Tou's T cells using a combination of a 5' VH4 leader sequence-specific primer (TL48) and a 3' end VH4 flanking region primer (TL 65). The results of these experiments demonstrated that the germline of patient Tou indeed contains a gene segment that differs by only one nucleotide from the sequence of the published VH4.21 gene segment. The one nucleotide difference was also present in the expressed VH4 gene in cell line T14, rendering it highly likely that the expressed VH4 gene was derived from this allele. Furthermore, none of the other 24 VH4 genes, including two new members of the VH4 gene family, displayed a higher degree of homology with the sequence of the mutated VH4 gene than VH4.21 (results summarized in Table 2). Similarly, we designed two primers hybridizing to the 3' and 5' sequences flanking the kv325 coding region (TL75 and TL52, Table 1; reference 29) to examine whether the nucleotide differences between the expressed VKIIIb gene segment in T14 and the published kv325 nucleotide sequence reflect true somatic mutations or a polymorphism of this VK gene segment. PCR-amplified material from genomic DNA extracted from the T cells of patient Tou was cloned into Bluescript and several independent VKIII-hybridizing colonies were selected for sequencing. The results of these experiments showed that the germline of patient Tou harbors a VKIIIb gene segment that shares 100% sequence homology with the published kv325 gene (Fig. 3). In line with these results, we found that T14 did not react with the anti-VKIIIb mAb 17.109 but did react with the anti-VKIIIb mAb JG-B1. The latter mAb is less affected by changes in the structure of the VKIIIb L chain as a result of somatic mutations (Carson, D., personal communication).

**Figure 5.** Comparison of the deduced amino acid sequences of the germ-line and expressed VH genes (A, Tou-VH4.21 and VH4T14) and the germ-line and expressed VL genes (B, Tou-vk325 and VKIIIb-T14) of cell line T14. The homologous germline VH and VL genes are derived from patient Tou's own germline DNA. Single-letter amino acid code is used. Dashes indicate identity with the germline sequence.

**Figure 6.** Alignments of nucleotide sequences of DH, JH, and JK elements expressed in cell line T14 and published germline elements (references 35 and 36). See legend to Fig. 2. The sequence data are available from EMBL/GenBank/DDBJ under accession numbers X56592 and X56594.
understanding of humoral autoimmunity. Indeed, analysis of these processes are imprinted in the Ig receptor molecules of the B cells that partake in these responses: polyclonally-activated B cells express germline V regions with no or a few randomly distributed somatic mutations whereas antigen-stimulated and selected B cells express V regions which harbor nonrandom distributions of somatic mutations (reviewed in reference 38). Thus, the molecular genetic analysis of the V regions of pathogenic autoantibodies may contribute to our understanding of humoral autoimmunity. Indeed, analysis of the V regions of autoantibodies secreted by hybridomas from MRL/lpr mice, has proven pivotal in understanding the pathogenesis of humoral autoimmunity in this animal model for human SLE (11, 12). These studies in the MRL/lpr mouse strongly suggest that anti-DNA autoantibodies arise as a consequence of an antigen-driven, oligoclonal B cell activation and expansion as manifested by the occurrence of nonrandomly distributed somatic mutations in the V regions of clonally-related IgG anti-DNA autoantibody-secreting hybridomas. Though serologically and pathologically similar to human SLE, the MRL/lpr mouse displays additional immunological abnormalities not normally associated with human SLE. Therefore, understanding the processes that lead to the generation of pathogenic autoantibodies in humans requires the analysis of the V regions of human B lineage cells that secrete IgG autoantibodies. Therefore, we investigated the binding characteristics, idiotypic expression, and complete nucleotide sequences of the expressed VH and VL genes and their germline counterparts of an IgG anti-DNA autoantibody derived from a patient with active SLE.

**Antigen Binding Properties of the IgG Anti-DNA Autoantibody.** Several properties of the T14 autoantibody distinguish it from previously described human monoclonal autoantibodies. T14 is a high affinity IgG anti-dsDNA autoantibody characteristic of serum and immune-complexed anti-DNA antibodies of patients with SLE. Moreover, T14 is of the IgG3 subclass and therefore able to fix complement. High affinity, complement fixing anti-DNA antibodies are thought to participate in the pathogenesis of glomerulonephritis in SLE patients and in a number of animal models for SLE (39-41). Unlike many IgM anti-DNA autoantibodies analyzed thus far (13-18), T14 does not belong to the class of multireactive antibodies as manifested by the lack of reactivity with a panel of 13 antigens. Together these properties of the T14 mAb render it highly likely that this antibody is involved in the disease process in this SLE patient.

**VH, DH, and JH Utilization.** The VH region expressed in cell line T14 is the rearrangement product of a member of the VH4 gene family, the DXP1 gene segment and the JH4 gene segment. Nucleotide sequence comparison with published VH4 genes suggested the use of a somatically mutated form of the VH4.21 germline gene. Alternatively, such nucleotide differences could stem from novel members and/or from polymorphisms of known members of the VH4 gene family. In order to discriminate between these alternatives, we obtained sequence data from 25 VH4 genes cloned from the patient's genomic DNA. Indeed, these data showed that the patient's VH4.21 gene displayed most homology with the expressed and mutated VH4.T14 gene. In this regard, a single base pair mismatch between patient Tou's expressed and germline VH.21 germline gene on the one hand and the published VH4.21 germline gene on the other hand was particularly informative. This strongly suggested that VH4.T14 was the mutated descendant of this allele, which was further supported by the finding that the patients germline DNA lacked the mutated CDR1 sequence present in VH4.T14. Together, these findings strongly suggest that the nucleotide substitutions in the VH regions that encode the T14 anti-DNA antibody result from somatic mutations.

A portion of the antigen-binding site of antibody molecules is encoded by the region that encompasses the DH element (CDR3). Recently, Cairns et al. (13) noted the frequent utilization of the germline DXP1 gene element in IgM anti-DNA autoantibodies from both patients and healthy individuals. In concordance with these results, we found expression of the DXP1 gene in the T14 IgG anti-DNA autoantibody. In each case DXP1 is used in the same reading frame which yields a total of 5 Tyrosine residues in this portion of the CDR3 region. Computer modeling has shown that the antibody combining site of IgG anti-DNA antibodies from NZB/NZW mice, an animal model for SLE, is dominated by arginine and tyrosine side chains that may contribute to DNA binding through electrostatic interactions (7). Similarly, a high tyrosine content of the CDR3 region of both natural and pathogenic human anti-DNA antibodies could be important in determining DNA binding activity. Moreover, these findings show a surprising degree of uniformity in the CDR3 region of some natural anti-DNA autoantibodies and the pathogenic anti-DNA autoantibody T14, suggesting a possible relationship between these two types of anti-DNA autoantibodies.

**VL and JK Usage.** The L chain expressed in cell line T14 is a somatically mutated form of the kv325 germline gene, a member of the VKIIIb gene family. We excluded the possibility that the VKIIIb.T14 gene segment was an allelic variant of kv325 by showing that the germline of patient Tou lacked the mutated sequence of VKIIIb.T14 and indeed contained an exact copy of the kv325 gene segment. Expression of the unmutated or mutated kv325 L chain in Ig molecules can be detected by the monoclonal antiidiotype antibody JG-B1. Indeed, T14 supernatant reacted with this mAb in an ELISA.

Several reports have shown that the kv325 gene, expressed in <1% of peripheral blood B cells, is preferentially expressed in κ-bearing CLL cells and small lymphocytic Non Hodgkin lymphoma, both neoplasms that commonly express the CD5 surface antigen (42, 43). Moreover, this particular VK gene segment is frequently associated with IgM paraproteins with autoantibody activity (44, 45) and was found in a multireac-
tive IgM anti-DNA/rheumatoid factor autoantibody from a leprosy patient (17). Further indirect support for a possible relationship between VKIIIb expression and autoreactivity in the CD5+ B cell population comes from the notion that both normal and malignant CD5+ B cells secrete autoantibodies after stimulation in vitro (46, 47) and the clinical observation that the sera of some patients with CLL contain autoantibodies and that 20% of these CLL patients develop autoimmune phenomena (48). In this context, the expression of a somatically mutated variant of kv325 in the IgG anti-DNA autoantibody T14 further suggests that this VK gene predisposes for autoreactivity, perhaps in combination with particular VH genes. Whether cell line T14 derived from a CD5+ B cell could not be established since many EBV-transformed cell lines lose CD5 expression after transformation. Indeed, T14 lacked CD5 expression at both the RNA and protein level.

Somatic Mutations in the T14 VH and VL Regions. The VH and VL genes expressed in the IgG anti-DNA-secreting cell line T14 differ by 14 and 5 nucleotides from the patients own germline VH4.21 and VKIIIb counterparts. The nucleotide differences are concentrated in the CDR regions and mainly lead to amino acid replacements. Such a nonrandom pattern of replacement mutations is highly suggestive of an antigen-driven B cell activation and selection process (12). Strikingly, the replacement mutations in CDR1 and CDR2 of both VH and VL yield a number of positively charged arginine and histidine residues. Additional arginine residues arose from replacement mutations of what presumably are nontemplated bases (N-regions) in the CDR3 of the VH region and from replacement mutations in FR3 and FR4. Interestingly, it has recently been suggested that a portion of the FR3 region (amino acids 67–85) may be involved in antigen binding (34). Indeed, the arginine introduced in FR3 falls within this stretch of amino acids. Arginine residues may interact with the phosphate moiety of the DNA backbone or form hydrogen bonds with guanine and cytidine groups in double stranded DNA and are important in promoting and/or stabilizing DNA-protein interactions (49). Indeed arginine residues have been shown to play an important role in determining specificity of anti-DNA antibodies from MRL/lpr and NZB × NZW mice (22).

The molecular analysis of the human IgG anti-DNA autoantibody T14 strongly suggests that, in analogy to murine models of SLE, at least part of the autoimmune response in SLE is (auto)antigen-driven. In the case of T14, this results in the generation of a high affinity anti-DNA autoantibody. This particular antibody utilized V and D elements that frequently encode low affinity IgM autoantibodies and have been repeatedly found in malignant B cells that express the CD5 antigen.

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