Evidence of Antigen Receptor-influenced Oligoclonal B Lymphocyte Expansion in the Synovium of a Patient with Longstanding Rheumatoid Arthritis

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Abstract

Plasma cell infiltration of synovium is common in longstanding rheumatoid arthritis (RA). The mechanism(s) underlying synovial B cell proliferation remains unclear. One theory invokes nonspecific polyclonal stimuli; another implicates antigen as the driving force. Antigen-driven repertoires are characteristically enriched for related sets of V gene segments containing similar sequence in the antigen binding site (complementarity-determining regions; CDRs). To study the forces shaping B cell proliferation, we analyzed Vκ transcripts expressed in the synovium of an RA patient. We found Humkv325, a developmentally regulated VκIII gene segment associated with autoantibody reactivity, in > 10% of randomly-chosen synovial Cκ cDNAs. Two sets of sequences contained identical charged amino acid residues at the Vκ–Jκ join, apparently due to N region addition. We generated "signature" oligonucleotides from these CDR3s and probed PCR amplified Vκ products from the synovium and PBLs of the same patient, and from PBLs and spleen of individuals without rheumatic disease. Significant expression of transcripts containing these unique CDR3 sequences occurred only in the patient's synovium. Thus, in this synovium there is expansion of a limited set of B cell clones expressing antigen receptors that bear evidence of antigen selection. (*J. Clin. Invest.* 1994. 93:361–370.) Key words: rheumatoid arthritis • immunoglobulins-kappa chain • genes-immunoglobulin • antibody diversity • B lymphocytes

Introduction

RA is an inflammatory disease involving the synovial membrane of diarthrodial joints (1, 2). Synovial plasma cells are relatively infrequent in the early stages, but increasingly prominent as the disease progresses. Indeed, local immunoglobulin production may reach levels equivalent to spleen (3, 4). The most common autoantibodies in RA are RFs, antibodies that bind the Fc portion of IgG. RFs can comprise up to 25% of synovial immunoglobulin (IgM, IgG, and IgA) in seropositive patients, i.e., patients exhibiting positive tests for serum RF, (4). Local production of RF is thought to participate in the pathogenesis of tissue injury in RA, although the exact mechanism of tissue damage is unclear (2, 5, 6).

Immunoglobulins are heterodimeric proteins consisting of two heavy and two light chains (7, 8). Each chain can be divided into a variable domain that defines the antigen specificity of the molecule, and a constant domain that is responsible for effector functions. Variable domains contain three intervals of sequence hypervariability, termed complementarity-determining regions (CDRs),[1] that are separated from each other by four intervals of relatively constant sequence, termed FRs. In man, κ light chain variable domains are assembled by splicing together one of more than 85 variable (V) gene segments (50 potentially functional) to one of five joining (J) gene segments (7, 9). The Vκ gene segment encodes CDRs 1 and 2, whereas CDR 3 is the product of Vκ–Jκ joining. The heavy and light chain CDRs are juxtaposed in the mature protein and form the antigen binding site (8).

We utilized a molecular cloning approach in order to examine the antibody repertoire expressed in the diseased synovium of a patient with longstanding, seropositive RA. Although the cloning strategies employed in this work do not permit us to directly correlate antibody sequence with antigen specificity, they have the compensatory advantage of allowing us to randomly sample the light chain repertoire at the site of tissue injury without the introduction of bias due to preferential cell growth under in vitro conditions. Transcript representation in a randomly primed cDNA library reflects mRNA abundance in the original tissue. At the terminal stages of differentiation, plasma cells may express 150–1000-fold greater immunoglobulin transcript levels per cell than their pre-B cell progenitors (10). Thus, cDNA library generation and screening enhances our ability to characterize the expression of transcripts derived from immunoglobulin-secreting cells within this synovium and to determine if there are clonal relationships among them.

Initial hybridization analysis of synovial transcripts suggested that the κ light chain repertoire might be enriched for transcripts containing the Humkv325 gene segment. The sequence of one of these Humkv325-containing clones, 10S2, has been previously reported (11). The Humkv325 Vκ gene segment is of particular interest since it is developmentally regulated (12) and is frequently expressed in the $CD5^+$ B cell population implicated in lymphoid malignancies (13, 14) and autoantibody expression (15). In germline or near-germline

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1. *Abbreviations used in this paper:* CDR, complementarity-determining region; CLL, chronic lymphocytic leukemia; CRI, cross reactive idiotype; FR, framework region; R/S ratio, replacement/silent substitution ratio; TdT, terminal deoxynucleotidyl transferase.

Table I. Oligonucleotide Probes and PCR Primers

Oligomer	Gene segment	Location	Orientation	Tm	Sequence (5′–3′)
				°C	
H-170*	Cκ	Cκ	anti-sense	74	CCAGAATTCAACTGCTCATCAGATGGCGCGGAAG
LSK-15*	Humkv325	FR 1	sense	68	GAAATTGTGTTGACGCAGTCTCCAGGCA
Actin 1	Actin		sense	64	CACAGAGCCTCGCCTTTG
Actin 2	Actin		anti-sense	54	TGGATAGCAACGTACATG
Actin 3*	Actin	internal		56	GCGATATCATCATCCATG
LSK-19	Cκ	Cκ	anti-sense	70	GCGCCGTCTAGAATTAACACTCTCCCCTGTTGAA
LSK-40	Vκ	Vκ leader	sense	69–92	GGGAATTCATGGACATG(AG)(AG)(AG)(AGT)(CT)CC(ACT)(ACG)G(CT)(GT)CA(CG)CTT
LSK-30	VκIII	FR 1	sense	71–87	GAAAT(ACT)(CG)T(AG)(AT)TGCA(AG)CACTCTCC(AG)(ACG)(CG)(AC)A(CT)C
LSK-16	Humkv325	leader/FR 1	sense	74	CCACCGCAGAGCTCGTGTTGACGCAGTCTCCA
LSK-1*	Cκ	Cκ	anti-sense	59	TGAAGACACATCGTGCAGCCA
LSK-23*	VκIII	FR 2	sense	71–84	TGGCCA(CG)(CAG)C(TG)CCCAG(GA)CTCCTC
LSK-25*	10S2	CDR 3	sense	69	CACTATGGTGGCTCGCCTCGGGAGTAC
LSK-33*	16S1	CDR 3	sense	53	CAGTATGGATATACACCCCTCACT
LSK-34*	28S1	CDR 3	sense	61	CAGTATCGTGACTCACCTCGGACG
LSK-37*	Humkv325-Jκ1	CDR 3	sense	64	CAGTATCGTAGCTCACCGTGGACGTTC

Columns show the oligonucleotide designation, the sequence from which the oligonucleotide is derived, the physical location of the oligonucleotide within its gene of origin, the orientation of the oligonucleotide with respect to the transcription, the calculated melting temperature for the oligonucleotide from its gene of origin, and the actual sequence of the oligonucleotide. Nucleotide designations in parentheses indicate sites of degeneracy, with equimolar amounts of each nucleotide. * denotes oligonucleotides used as probes; others were used as PCR primers.

form, the Humkv325 gene segment can be found in IgMκ paraproteins with RF activity (16, 17), as well as in antibodies with anti-intermediate filament activity (18), anti–single-stranded DNA activity (19), and anti-LDL activity (20). This association between the Humkv325 gene segment and autoreactivity led us to focus on the Humkv325-containing repertoire within this synovial sample.

In this study, we identified seven additional Humkv325-containing transcripts from a random sampling of 40 $C\kappa^+$ synovial transcripts. We found a repertoire of mutated Humkv325-containing light chains that was enriched for highly charged CDR 3 regions. Among these clones were transcripts that had likely arisen from the same B cell progenitor, as well as transcripts that shared identical CDR 3 deduced amino acid sequence yet were derived from different B cell ancestors. Using a PCR-based strategy, we tested the synovium and the peripheral blood of our patient and demonstrated that B cells expressing these κ chains were prominent only in the synovium. These findings suggest that the expansion of B cells in this autoimmune disease is likely the product of local antigen receptor-driven selection.

Methods

Patient characteristics, tissue isolation and cDNA library preparation. The clinical characteristics of our 62-yr-old female patient, B.C., and the methods used to process involved synovial tissue have been reported previously (11). Two oligo d(T)-primed cDNA libraries in λgt10 were independently generated from separate 2-μg aliquots of poly $(A)^+$ RNA from the same synovial tissue sample of this patient.

Identification of Humkv325-containing cDNA clones. An aliquot of 260,000 recombinants derived from the second cDNA library was screened with H-170, a modified 5′ Cκ anti-sense oligonucleotide (Table I). Each positive plaque was numbered sequentially and a random number generator was used to select six clones for random sequence analysis. The nitrocellulose filters were boiled to remove the radioactive probe, and then subsequently screened with a Humkv325 FR 1 specific oligomer, LSK-15 (Table I), at a hybridization stringency of 57°C in SSC-1% SDS (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7). All the clones that hybridized with the Humkv325-specific probe were cloned into pUC 19 and double-stranded sequencing was performed as previously described (11).

Molecular modeling. The κ light chain variable domains of clones 10S2 and 28S1 were modeled on an IRIS 4D/220GTX computer system (Silicon Graphics, Palo Alto, CA) using the molecular modeling software package QUANTA (Polygen, Waltham, MA). The crystallographic structure of mouse IgAκ J539 (File 2FBJ; 2.6-Å resolution; reference 21) was obtained from the Brookhaven database (22, 23). The mouse κ light chain variable domain was first altered to form a germline Humkv325-Jκ1 domain and then subsequently mutated to the sequence of 10S2 and 28S1, respectively.

Isolation of lymphocytes from peripheral blood and spleen. Peripheral blood was obtained from patient B.C. within 24 h of joint arthroplasty, and from a normal 32-yr old male volunteer (L.B.). Cadaveric spleen was obtained from a 49-yr-old white male, who was without a history of autoimmune disease and had died of congestive heart failure. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation (24). The guanidinium isothiocyanate technique was used to isolate total RNA from each sample (25).

PCR amplification of Vκ-containing transcripts obtained from total RNA from PBL, spleen, and synovium. Using the Superscript RNase H/MMV Reverse Transcriptase kit (BRL, Gaithersburg, MD), oligo d(T) primed first strand cDNA was generated from 10-μg samples of total RNA obtained from PBLs of patient B.C., PBLs of volunteer L.B., cadaveric spleen cells, and synovial cells of patient B.C. Reaction con-

ditions were as described by the manufacturer. Each cDNA sample was divided into 10 aliquots of equal size.

Individual PCR amplifications were performed on a 1-μl aliquot of first strand cDNA from each sample. The efficacy of cDNA preparation was measured by amplification for β actin (26). Primers for amplifying actin (Actin 1 and 2) and for hybridization (Actin 3) are listed in Table I. PCR conditions were: 25 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 4 min, and final extension at 72°C for 10 min.

In conjunction with an anti-sense Cκ oligonucleotide (LSK-19, Table I), Vκ-containing transcripts were amplified with a degenerate sense oligonucleotide (LSK-40) derived from the leader sequence of all known Vκ gene segments (27). In the spleen sample, the anti-sense Cκ oligonucleotide H-170, located 5′ to LSK-19, was used instead. VκIII-containing transcripts were amplified with LSK-19 and a degenerate sense oligonucleotide (LSK-30) derived from FR 1 of all known VκIII gene segments. Humkv325-derived transcripts were amplified with LSK-19 and a sense oligonucleotide LSK-16, derived from the leader and framework I of germline Humkv325. Each oligonucleotide contained minor 5′ terminal modifications to encode restriction sites for cloning. PCR conditions for each of these reactions were: 25 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 4 min, and final extension at 72°C for 10 min. In order to control for possible contamination, mock reaction mixtures lacking template or lacking reverse transcriptase were prepared. None of the controls contained amplified products.

Southern blot analysis of PCR amplification products. One-fifth aliquots of each amplified product were electrophoresed through 1.5% agarose gels and blotted onto Nytran (Schleicher and Schuell, Keene, NH) membrane filters. Ethidium bromide staining was used to verify that approximately equal amounts of DNA had been loaded into individual wells. After UV cross-linking, filters were prehybridized at 37°C in a solution containing Southern blot buffer (1 M NaCl, 0.2 M Tris-HCl, pH 7.5, 0.1% SDS, 10X Denhardt's solution) and 0.5 mg of salmon sperm DNA. Oligonucleotide probes (Table I) were end labeled with $\gamma[^{32}P]$ATP and polynucleotide kinase to a specific activity of 1×10^6 to 5×10^7 cpm/ml of hybridization solution. The filters were hybridized for 12–16 h at 37°C in Southern blot buffer in the presence of 0.5 mg of salmon sperm DNA. Hybridization stringency was determined at the time of washing. Filters were washed twice in SSC–1% SDS at room temperature for 5 min, then overnight at 42°C. Autoradiograms were generated by exposing the filters at −70°C for 30 min to 4 d with intensifying screens. After the initial exposure, the filters were washed again overnight at a temperature 5°C below the calculated melting temperature of the oligonucleotide probe (Table I). After each final exposure, the filters were stripped by boiling for 30 min. Each filter was probed sequentially with the following oligomers: LSK-1 (internal Cκ), LSK-23 (FR 2 VκIII), LSK-25 (10S2-like CDR 3), LSK-33 (16S1-like CDR 3), LSK34 (28S1-like CDR 3), and LSK-37 (germline Humkv325-Jκ1 CDR 3). A control filter containing clones 10S2, 16S1, 25S4, 25S5, 28S1, 37S4, and pUC19 dotted onto a Nytran filter was prepared as above.

Results

Cloning and identification of seven Humkv325-derived transcripts from synovium. In our previous analysis of 24 Cκ^+ transcripts cloned from dissociated synovial cells from this patient, we identified a single Humkv325-containing clone, 10S2, among the 15 cDNAs that contained Vκ sequence (11). Because this initial library suffered from incomplete EcoR I methylation, another aliquot of synovial-derived poly (A)$^+$ RNA from the same tissue sample was used to independently generate a second library. In this second library, 40 Cκ^+ clones were identified among 260,000 recombinants. The success of the methylation procedure was verified by sequencing six randomly selected recombinants. Two of these recombinants (clones 37S4 and 38S1) were found to contain mutated Humkv325 gene segments (Fig. 1). The remaining 34 Cκ^+ recombinants were screened with a FR 1 Humkv325-specific oligonucleotide (LSK-15, Table I). Five additional Humkv325-derived clones were identified and sequenced, making a total of seven Humkv325-derived clones, in addition to clone 10S2 from the original analysis (Fig. 1). Their sequences were compared to the known germline Vκ repertoire (28–30) and their likely Humkv325 germline origin confirmed by parsimony.

Oligoclonal expansion of B cells expressing 10S2-like κ light chains. The Humkv325 gene segment represents only one of 50 potentially functional Vκ gene segments in the haploid genome, yet Humkv325 was used by at least 12.5% (8 of 64) of this random sampling of synovial κ transcripts ($P < 0.00004$, binomial distribution). Evidence of clonal relatedness was sought by comparing the sequences of these Humkv325-containing cDNAs to each other (Fig. 1). From the leader to the terminus of the Jκ sequence, two clones, 21S2 and 38S1, were base for base identical to the Humkv325 clone, 10S2, that we had previously identified from the first library (11).

Although derived from different cDNA libraries, the 5′ termini of clones 10S2 and 21S2 are identical. The identity of these sequences did not allow us to definitively distinguish between the presence of a preferred reverse transcriptase stop site or possible contamination of the second library with 10S2 sequences. However, there is no question that 38S1 is a unique clone because the 5′ sequence extends beyond the site of reverse transcriptase termination in the other two (Fig. 2). Out of a total of 64 Cκ^+ cDNA transcripts, three (5%) contained 10S2-like sequences. Thus, B cells bearing 10S2-like transcripts are over-represented in the synovium of this patient.

Replacement/silent substitution ratio analysis of the Humkv325-derived sequences. Insight into the forces that have shaped the products of somatic mutation can be gained from analysis of the amino acids specified by the altered codons. Nucleotide changes in a codon may result in either a replacement (R) of the amino acid by another or preservation of the same residue, termed a silent (S) mutation. Codons undergoing random mutation are predicted to yield a replacement/silent substitution (R/S) ratio of 2.9 (31, 32). Selection for conservation of protein sequence yields R/S ratios of less than 2.9, whereas selection for diversity results in ratios significantly greater than 2.9. For example, antibodies elaborated during a secondary response to antigen are associated with R/S ratios in the CDRs significantly greater than 2.9, indicating positive selection for high affinity antigen binding sites (32–34). The average R/S ratio in the framework regions of the 8 Humkv325-derived variable domains was 0.8 (FR 1, 0.3; FR 2, 0.7; FR 3, 1.1; FR 4, 0.5), indicating preservation of essential function.

Among the eight sequences, there were eight replacement mutations in CDR 2 giving an R/S ratio of 8.0. The R/S ratio for CDR 1 was 3.0 and for CDR 3 was 1.7, yielding an average R/S ratio of only 3.0. Although this pattern initially suggested random mutation in the CDRs, closer inspection revealed numerous shared or conserved amino acid changes among these sequences. For example, six of the eight clones had mutations resulting in changes from serine to asparagine at position 31 and both the 10S2-like sequences (10S2, 21S2, and 38S1) and clone 28S1 contained a threonine at position 29. Even more

A Nucleotide Sequences

```
          |<--                        Framework I                        -->|
            1          5          10         15          20
Humkv325  GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGC
**10S2    .....................................................................
 *21S2    .....................................................................
 *38S1    .....................................................................
  28S1    .....................................................................
  37S4    .....................................................................
  25S5    .......................G...........................G.................
  25S4    .....................................................G...............
  16S1    ........A............................................................

          |<--          CDR 1         -->|<--          Framework II           -->|<--   CDR 2     -->|
               25    27A 28   30        34 35          40             45            50          55
Humkv325  AGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCC TGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT GGTGCATCCAGCAGGGCCACT
**10S2    ...............C...C.....A.......... ................................A............ ....T................
 *21S2    ...............C...C.....A.......... ................................A............ ....T................
 *38S1    ...............C...C.....A.......... ................................A............ ....T................
  28S1    ...................C.TA..A...T....G. ......................G...................... .....................
  37S4    ........................G........... ............................................. .C...................
  25S5    ............TA.T..G..TC..A.......... ........A.....................C........G..... .....................
  25S4    ....................TTAT..T...C.G... ............................................. ...A.C...............
  16S1    ...........A.AGA...AG.A..A...T...... ................................C....G...A... ....T.....T..........

          |<--                                   Framework III                                        -->|
            57     60             65             70             75             80             85      88
Humkv325  GGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGT
**10S2    ............................C..............................T................................T...
 *21S2    ............................C..............................T................................T...
 *38S1    ............................C..............................T................................T...
  28S1    ................................................................................................
  37S4    ................................................................................................
  25S5    .................T.T........T.....T.......................C.C.......................A.A.........
  25S4    ...............G....................................................................A...........
  16S1    ..................................A.....................T..T....................................
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B Deduced Amino Acid Sequences

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          |<--   Framework I   -->|<--CDR 1 -->|<--  FW II   -->|<CDR 2>|<--        Framework III        -->|
          1        10        20         30         40          50      60        70        80
Humkv325  EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
**10S2    ....................... .....LT.N... ............... .V..... .........A......................
 *21S2    ....................... .....LT.N... ............... .V..... .........A......................
 *38S1    ....................... .....LT.N... ............... .V..... .........A......................
  28S1    ....................... ......TYN..G .......R....... ....... ................................
  37S4    ....................... ........G... ............... A...... ................................
  25S5    .................G..... ....YFG.N... .............V. ....... ......I..V.V.......T........I...
  25S4    ....................... .......Y.... ............... GT..... .....V......................M...
  16S1    ....................... ....KIKNN... ..........S.V.. .V.I... ...........E....................
```

C Nucleotide Sequences of CDR 3 Regions

```
          |<--          CDR 3                 -->|<--         FR 4         -->|
           89 90 91 92 93 94 95    95A95B    96 97  98 99 100              107
Humkv325  CAGCAGTATGGTAGCTCACCTCC|<--N--->|<--                Jκ             -->|
**10S2    .....C......G....G....    GGGA    ....... .............................. Jκ2
 *21S2    .....C......G....G....    GGGA    ....... .............................. Jκ2
 *38S1    .....C......G....G....    GGGA    ....... .............................. Jκ2
  28S1    ............GA........              ..... .............................. Jκ1
  37S4    ............GA...C..       AA       ..... .............................. Jκ1
  25S5    ..T.G......A.C.........           ....... .............................. Jκ5
  25S4    ..............T...T.G              ...... .......................A....G. Jκ2
  16S1    ..C........ATATA....C.              ..... ........C..................... Jκ4
```

Figure 1. (*A*) Nucleotide sequences of the eight Humkv325-derived light chain transcripts from RA synovium. The top line is the germline Humkv325 sequence (16, 17). All clones listed below it are compared to this sequence, with a dot denoting homology and a letter indicating a nucleotide difference. The numbers above the Humkv325 sequence indicate the codon numbers. Clone 10S2 (**) is the Humkv325-derived clone found in the first synovial library from this patient and reported previously (11). Clones 21S2 and 38S1 (*) are identical in nucleotide sequence to clone 10S2. (*B*) The deduced amino acid products of the germline element and the cDNA clones are presented in single-letter code with a dot denoting identity to the germline progenitor. Shared sequence changes in the CDR 1 region of clones 10S2, 21S2, 38S1, and 28S1 are underlined. (*C*) Nucleotide sequences of CDR 3 regions of eight Humkv325-derived clones are compared to the germline Humkv325 sequence and to the corresponding Jκ sequence, with a dot denoting germline identity. Nucleotides at the Vκ–Jκ junction, which could not be assigned to either the Vκ or Jκ gene segments, are identified as the likely products of N region insertion. (*D*) The deduced amino acid products of each germline element and cDNA clones are presented in single-letter code with a dot denoting identity to the germline progenitor, or to corresponding Jκ. As a result of N region addition, the CDR 3 regions of 10S2, 21S2, and 38S1 are 11 amino acids in length. The GenBank accession numbers for these sequences are: 1651–U03480, 2554–U03481, 2555–U03484, 2851–U03482, and 374–U03483. The EMBL accession number for 1052 is X58081.

D Deduced Amino Acid Sequences of CDR 3 Regions

	CDR 3											FR 4										
	89	90	91	92	93	94	95	95A	95B	96	97	98	99	100	101	102	103	104	105	106	107	
Humkv325	Q	Q	Y	G	S	S	P			|<--					Jκ						-->|	
**10S2	.	H	.	.	G	.	.	R	E	.	.	.	.	.	.	.	.	.	.	.	.	Jκ2
*21S2	.	H	.	.	G	.	.	R	E	.	.	.	.	.	.	.	.	.	.	.	.	Jκ2
*38S1	.	H	.	.	G	.	.	R	E	.	.	.	.	.	.	.	.	.	.	.	.	Jκ2
28S1	.	.	.	.	D	.	.			R	.	.	.	.	.	.	.	.	.	.	.	Jκ1
37S4	.	.	.	.	D	.	.	R			.	.	.	.	.	.	.	.	.	.	.	Jκ1
25S5	H	R	.	.	T	.	.	P		.	.	.	.	.	.	.	.	.	.	.	.	Jκ5
25S4	.	.	.	.	.	.	S			.	.	.	.	.	.	.	.	.	.	.	R	Jκ2
16S1	H	.	.	.	Y	T	.			.	.	.	.	A	.	.	.	.	.	.	.	Jκ4

Figure 1. (Continued)

revealing was an analysis of the amino acid composition of the CDR 3 regions.

Analysis of the CDR 3 region discloses selection for charged amino acids. Of the 24 amino acid changes from germline sequence in the CDR 3 region, two thirds involve the insertion of charged amino acids: histidine, aspartic acid, glutamic acid, and arginine ($P < 0.00002$, binomial distribution). Among those sequences with N region addition (10S2, 21S2, 38S1, and 37S4), all contain an arginine at the Vκ–Jκ junction, including the 3 with 11 amino acids in CDR 3.

In clone 28S1, the Vκ–Jκ junctional arginine was generated by germline sequence, whereas the remaining charged amino acids appear to be the products of somatic mutation. Although clone 28S1 does not contain N nucleotides, it shares identical CDR 3 amino acid sequence with clone 37S4, including an arginine at the Vκ–Jκ junction. Closer inspection of clone 28S1 reveals further similarity to the 10S2-like sequences. Among the nine replacement mutations in clone 10S2, three of the amino acid substitutions are also present in clone 28S1: a Ser to Thr substitution at position 29 in CDR 1 and a Ser to Asn at position 31 in CDR 1, as well as the arginine at the Vκ–Jκ junction.

10S2-like sequences are over-expressed in synovium, but not in peripheral blood. The 10S2-like sequences contain four bases of N nucleotides at the Vκ–Jκ junction. The N nucleotides, in conjunction with somatic mutations in the Vκ CDR 3, create a sequence "signature" that uniquely identifies clones with this Vκ–Jκ join. We performed identical PCR amplifications on cDNA from the RA patient's synovium, her PBLs (obtained within 24 h of joint arthroplasty), PBLs of a normal 32-yr-old volunteer, and mononuclear cells derived from the cadaveric spleen of a 49-yr-old male. Ethidium bromide staining revealed roughly equivalent amplification of Vκ transcripts in all the tissues tested, a finding confirmed by hybridization with internal oligonucleotides (Fig. 3 *A* and *B*). When the PCR products amplified with Humkv325 and Cκ primers were probed with the 10S2 signature oligomer LSK-25, the 10S2-like Vκ–Jκ join was detected only in the synovium of our patient (Fig. 3 *D*).

Hybridization analysis of PCR amplification products reveals enrichment in the synovium for a second set of sequences with charged CDR 3 domains. To determine whether or not 28S1-like sequences were also over-represented in synovium, we generated a signature oligonucleotide probe (LSK-34) from the CDR 3 sequence of clone 28S1. This probe was hybridized to our PCR amplified Vκ-transcripts (Fig. 4). Autoradiography revealed enrichment for 28S1-like sequences in synovium, but not in the patient's peripheral blood nor in our normal controls.

Clone 16S1 was unique among these synovial clones in that it did not contain an arginine residue in either CDR 3 or FR 4. We generated a signature oligonucleotide probe (LSK-33) from the CDR 3 sequence of 16S1 and hybridized it to our PCR amplified Vκ-transcripts (Fig. 4). Unlike the two charged CDR 3 domains, the mutated CDR 3 of 16S1 was expressed at relatively low levels in all samples tested.

To test for the presence of more commonly reported Vκ–Jκ junctions (nine amino acid CDR 3 and no N region addition), we generated an oligonucleotide signature sequence complementary to a nine amino acid germline Humkv325-Jκ1 CDR 3 junction (LSK-37). Hybridization of this oligonucleotide to our panel of Vκ PCR amplified products revealed the presence of Vκ–Jκ rearrangements of this type in all tissues tested, although the level of these rearrangements appeared somewhat lower in the tissues of our patient than in our controls. Thus, it appears that VκIII-containing transcripts from this synovial sample, in contrast to those in normal spleen and PBL, are enriched for CDR 3 regions resembling 28S1 as well as 10S2.

Molecular modeling of the 10S2-like and 28S1-like sequences. To analyze the contribution of the shared changes in CDR 1 (codons 29 and 31) and CDR 3 (arginine at codons 95A and 96) between the 10S2- and 28S1-like sequences, we modeled the κ light chain on the mouse IgAκ J539 structure (Fig. 5). The shared CDR 1 mutation at codon 29 is predicted

Nucleotide Sequence of 5' Untranslated Regions of Identical Clones

```
                |<--5'Untranslated region-->|
Humkv325:   //GCTCAGTTAGGACCCAGACGGAACC
    10S2:                 *.....G......
    21S2:                 *.....G......
    38S1:    *.................G......
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Figure 2. The 5′ terminal sequence of clones 10S2 (11), 21S2, and 38S1. Nucleotide sequences are compared to the germline sequence of the 5′ untranslated region of Humkv325, with a dot denoting germline identity. *Denotes reverse transcriptase termination site. Clone 10S2 is the Humkv325-derived clone found in the first synovial library from this patient (11).

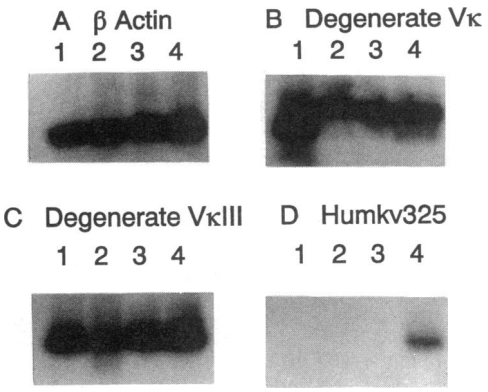

Figure 3. Autoradiographs from Southern blot hybridization of PCR products. Amplifications were performed on first strand cDNA generated from the tissues listed below. 20 μl of amplification products were electrophoresed on 1% agarose and transferred to nitrocellulose by standard protocols. In each panel, lane *1* contains amplification products from spleen cells of a 49-yr-old white male without autoimmune disease, lane *2* from PBLs of a normal 32-yr-old white male, lane *3* from PBLs of a 62-yr-old white female with RA, and lane *4* from synovial tissue of the same 62-yr-old white female with RA. (*A*) PCR amplification of cDNA for β actin, using primers Actin 1 and Actin 2 and probed with an internal oligonucleotide, Actin 3 (Table I). All reactions yielded a band of expected size, 498 bp. (*B*) PCR amplification of cDNA for the majority of Vκ transcripts, using a degenerate leader primer (LSK-40) and a Cκ primer (LSK-19), and probed with an internal Cκ probe, LSK-1 (Table I). Lanes *2, 3,* and *4* show bands of expected size, ~ 700 bp. The smaller size of the band in lane *1* is due to use of a different Cκ primer (H-170, Table I), located 249 bp 5′ to LSK-19. (*C*). PCR amplification of cDNA for the majority of VκIII transcripts, using a degenerate FR 1 primer (LSK-30) and a Cκ primer (LSK-19), and probed with a VκIII FR 2 oligonucleotide (LSK-23) (Table I). All reactions yielded bands of the expected size, ~ 625 bp. (*D*) PCR amplification of cDNA for the majority of Humkv325-derived transcripts, using a leader-FR 1 primer (LSK-16) and a Cκ primer (LSK-19), and probed with an oligonucleotide specific for the CDR 3 of clone 10S2 (LSK-25). Agarose gel electrophoresis showed bands of expected size, ~ 625 bp. Only the band in lane *4*, from RA synovium, hybridized to the 10S2 CDR 3-specific probe.

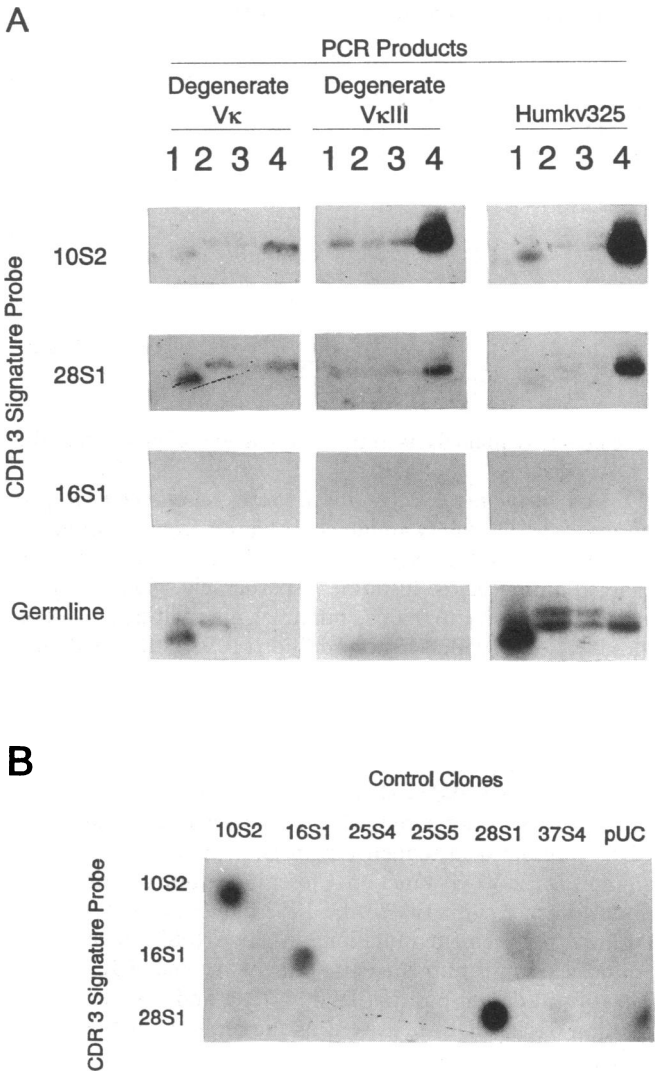

Figure 4. (*A*) Autoradiographs of Southern blot hybridization of PCR products probed with four different CDR 3 "signature" probes derived from clones of interest. Lanes *1–4* contain PCR products from the same reactions described in Fig. 3. Probes used were specific for Vκ–Jκ joins of clone 10S2 (LSK-25), clone 16S1 (LSK-33), clone 28S1 (LSK-34), and for the Vκ–Jκ join of germline Humkv325 to Jκ1 (LKS-24) (Table I). As in Fig. 3, the smaller size of bands in lane *1* in degenerate Vκ and Humkv325 amplifications is due to use of a more 5′ Cκ primer, H-170. (*B*) Control strip containing clones with different CDR 3 regions (Fig. 1 *C*), hybridized with probes LSK-25, LSK-33, and LSK-34, demonstrating specificity of probes. Washing was done at 60°C in SSC–1% SDS. After washing, the filter was exposed to x-ray film with an intensifying screen for 7 d at − 70°C.

to be solvent-exposed and thus potentially capable of interacting directly with antigen. However, the extent of exposure of residue 31 may depend on the structure of the CDR 3 region of the V_H gene segment. In contrast, amino acid 96, the most somatically variable residue during initial light chain generation, was not solvent-exposed, but rather buried beneath the surface of the antigen binding site.

Discussion

Using a combination of cDNA library (11) and PCR amplification approaches, we have demonstrated over-representation of Humkv325-containing transcripts in the involved synovium of a patient with longstanding RA. Furthermore, among these transcripts there is strong evidence of oligoclonal expansion of B cells expressing light chains with similar sequence in the CDRs, which encode the antigen binding site. These data support our previous conclusion that B cell expansion in this synovium is likely the product of specific antigenic stimulation. Other salient features include an unusually high rate of N region addition, the presence of charged amino acids in the CDR 3 regions, and many long CDR 3 regions. These abnormal findings further suggest the possibility of abnormal regulation of immunoglobulin gene expression in this tissue.

Recognition of antigen results in B cell expansion and differentiation into antibody-secreting plasma cells. Within the germinal centers found in normal spleen, antigen-responsive B cells undergo oligoclonal proliferation and somatic hypermutation (35). Initially, somatic mutations appear scattered at random throughout the variable domain. With maturation of the response, there is selection for variable domains that have

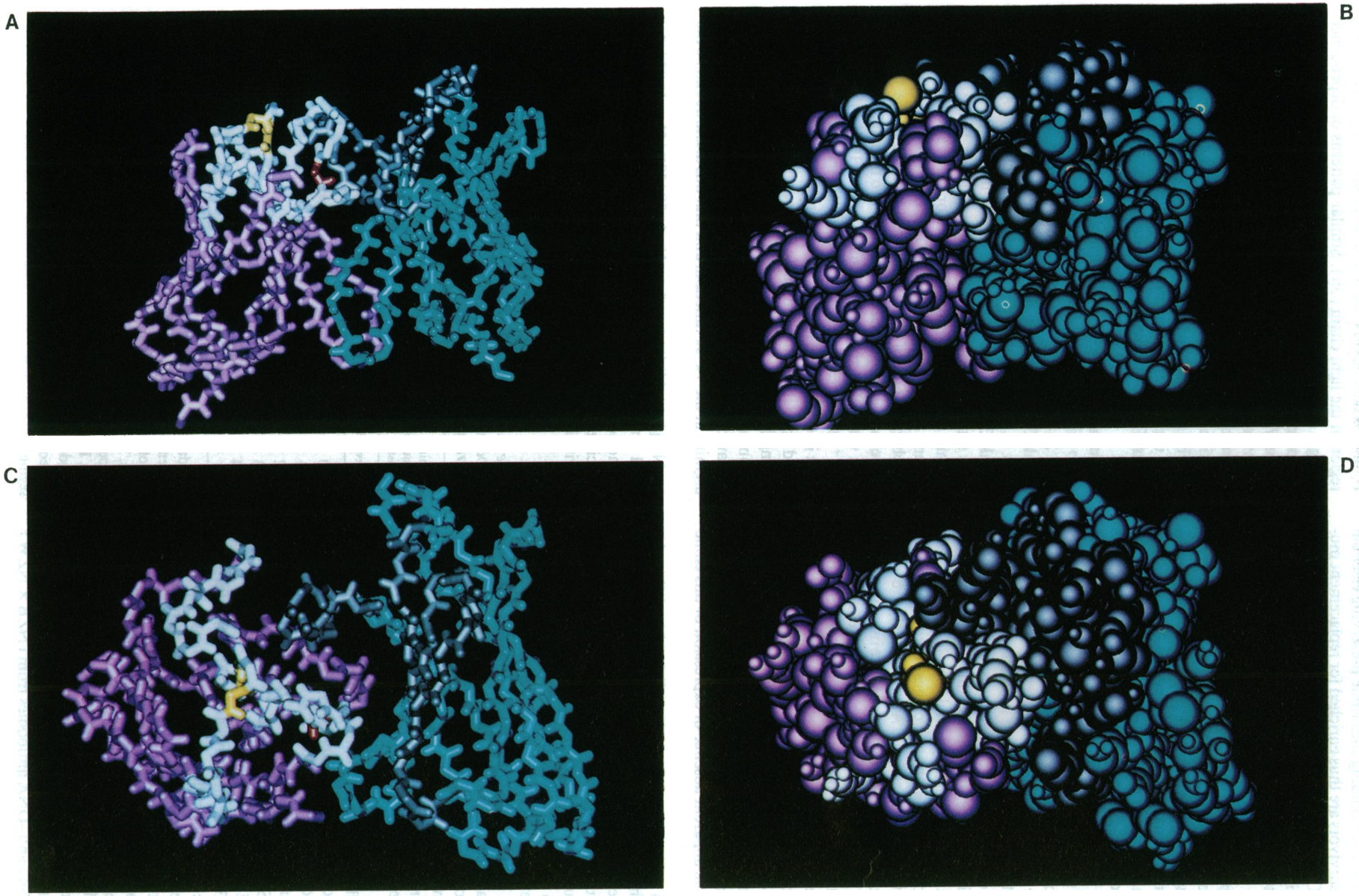

Figure 5. Predicted structure of the heavy and light chain variable domains of the 10S2 molecule. Frameworks 1, 2, and 3 of the light chain are shown in violet and frameworks 1, 2, and 3 of the heavy chain are shown in blue. All three CDRs of the light chain are white; the three CDRs of the heavy chain are dark gray. The V_κ–J_κ junctional residue 96 is colored red. The somatic mutation at CDR 1 residues 29 and 31 are shown in yellow; these amino acid changes are shared between clones 10S2 and 28S1 (Fig. 1 *B*). (*A*) Backbone-only view and (*B*) three-dimensional raster generation of the structure as viewed from the side with the antigen binding site at the top. (*C*) Backbone-only view and (*D*) three-dimensional raster generation of the structure as viewed fron the antigen binding site at the top. The red of residue 96 can be seen buried beneath other CDR residues in (*A*) and (*C*); (*B*) and (*D*) show that residue 96 is hidden from the surface. Residue 29 is clearly solvent-exposed when viewed from either perspective. The extent of exposure of residue 31 depends on the sequence and structure of the V_H CDR 3 region.

generated novel antigen binding sites while preserving essential structure. The survivors are thus enriched for replacement mutations in the CDR regions. Thus, although the initial repertoire may be highly diverse, the final repertoire can be dominated by a limited population of sequences derived from different progenitor clones that by chance express the preferred canonical antigen binding sites (35).

A similar focusing of the immune response has also been described in murine models of SLE. The anti-DNA associated idiotype that predominates in the late stages is not readily apparent early in the course of the disease. With time and the progression of disease, enhanced specificity for antigen results in selection for heavy chains that bear arginine residues in the CDR 3 domain. Thus, nonclonally related sequences often share similar somatic mutational changes (36, 37).

The unique sequence of the CDR 3 region of clones 10S2, 21S2, and 38S1 suggests they are the product of a single gene rearrangement event. These three sequences represent ~ 5% of the 64 Cκ positive clones identified in the two unrestricted cDNA libraries from this single synovial sample. PCR analysis confirmed the over-representation of clones bearing these rearrangements in this synovial sample. Approximately 1.6×10^6 cells in this synovial sample were able to secrete immunoglobulin in culture (11). Although it is possible that clones 10S2, 21S2, and 38S1 all arose from the same plasma cell, it is more likely that cells expressing this light chain represent an oligoclonally expanded population of B cells. Such an oligoclonal expansion could be due either to neoplastic transformation or to antigen-driven expansion. To distinguish between these possibilities, we examined the PBLs of the patient for the presence of this clone. The apparent absence of expression of this clone in her blood lymphocytes, and the lack of evidence of a neoplastic process over a 3-yr follow-up period, suggest that the expansion of this clone is a local event. These results are compatible with the hypothesis that RA can be a polycentric disease in which immune events at local sites of tissue inflammation are autonomously regulated (38).

In addition to the 10S2-like sequences, this synovial sample also contains evidence of oligoclonal expansion of B cells expressing other Humkv325-derived κ light chains. Clone 37S4 shares identical CDR 3 deduced amino acid sequence with clone 28S1. Clone 28S1 is the product of a typical germline join while 37S4 contains non-germline-encoded nucleotides at the Vκ–Jκ junction, suggesting convergent evolution of the immune response. Clones 28S1 and 10S2 share sequence similarity in the CDR 1 and 2 regions, but the sequences of the CDR 3 regions are different, both in number of amino acid residues and in Jκ usage (Fig. 1). Thus, there could potentially be several sets of oligoclonally expanded B cells in this RA synovium. The shared mutations between apparently nonclonally related transcripts 10S2 and 28S1 are similar to findings in murine models of SLE (36, 37).

The CDR 3 regions of the heavy and light chains form the center of the classically defined antigen binding pocket (39). Changes in the sequence of the Vκ–Jκ junction introduced by both genetic and somatic mechanisms may thus affect antigen binding and specificity. For example, anti-galactan antibodies are enriched for light chains with isoleucine at the Vκ–Jκ junction (40). mAbs raised in A/J mice against the hapten *p*-azophenylarsonate all contain an arginine at position 96, which is essential for antigen binding (41). Similarly, the specificity of both IgM and IgG anti-DNA antibodies from (NZB × NZW) F_1 mice is dependent on arginine in position 96 of the CDR 3 region of the light chain (36). Similar patterns of dependence on light chain junctional diversity are seen in man. The most common light chain components of human IgG antibodies against *Haemophilus influenzae* type b polysaccharide contain an invariant arginine at the Vκ–Jκ junction (42). Therefore, expansion of B cell clones with unusual sequences at the Vκ–Jκ junction is often associated with antigen selection.

The potential diversity of Vκ–Jκ splice sites is much less than is possible in immunoglobulin heavy chain variable domains. The overwhelming dominance of nine amino acid CDR 3 regions, the absence of N region addition in light chains, and the absence of diversity (D) gene segments contribute to this difference. A nine amino acid CDR 3 region predicts only 21 different possible nucleotide sequence products of Humkv325-Jκ rearrangements, encoding eight different amino acids at position 96, the splice site (Table II). Among 36 Humkv325 sequences reported in the literature (8, 43, 44), none share sequence identity with our clones. Four sequences contain an arginine at position 95A or 96 in association with Jκ1 and one in association with Jκ2. One clone was from the peripheral blood of a normal individual (43), one from an IgM-expressing CLL cell line (45) and two from IgMκ cryoglobulins with RF activity (8). Finally, clone HEW (45), from a $CD5^+$ B cell expressing the 17.109 CRI, contains 10 amino acids in CDR 3 and likely is the product of N region addition. Based upon the analysis in Table II, the arginine in the Jκ2-containing IgMκ cryoglobulin must be the product of either N region addition or somatic mutation. These findings indicate that autoantibodies with specificities found in RA may express arginine at the Vκ–Jκ junction, and that this arginine can be the product of somatic events.

N region addition was found in 4 of 8 (50%) Humkv325-derived transcripts from this synovial sample, leading to 11 amino acid CDR 3 regions in three clones. N region addition is associated with the activity of the enzyme TdT (46, 47). TdT activity is only present in B lineage cells before production of a

Table II. Analysis of the Potential Sequence Diversity of Codon 96 in Humkv325-Jκ Rearrangement in the Absence of N Region Addition

Terminal Nucleotides Lost	Vκ–Jκ Junction: Amino Acid at Position 96				
Humkv325	Jκ1	Jκ2	Jκ3	Jκ4	Jκ5
0	P	P	P	P	P
1	R	H	L	L	L
2	W	Y	F	L	I
3	W	Y	F	L	I
4		Y			

Standard one-letter abbreviations for amino acids are shown. Rearrangement without terminal nucleotide loss results in the introduction of a proline residue at position 95A and a CDR 3 of ten amino acids. To ensure a CDR 3 length of nine amino acids, there must be compensatory nucleotide loss in either the Vκ or Jκ gene segment, or both. Listed is the expected amino acid residue one-letter abbreviation at codon 96 for each Jκ gene segment in relation to the number of nucleotides removed from the 3′ terminus of the Humkv325 gene segment.

functional heavy chain (46, 47). Somatic nucleotide insertions that increase the size of the light chain CDR 3 domain have been reported as a result of experimental manipulation or abnormal recombination (48–50). In addition, N region addition has been found in a high proportion of VκIII-containing RFs from RA patients (51). Presumably these are situations in which light chain rearrangement has occurred in the presence of TdT activity. Sequence analysis of the light chains of RFs derived from both normal individuals and patients with rheumatologic disorders revealed that two of nine light chains contained 10 and 11 amino acids in the CDR 3 region, respectively (44). Both these RFs and the transcripts described in this report may derive from unusual progenitor B cells that failed to regulate TdT expression appropriately. We may speculate that such B cells could be more prone to escape other forms of normal regulation and thus be at greater risk for producing potentially deleterious antibodies.

Molecular modeling of the 10S2-like and 28S1-like sequences demonstrates that residue 29 in CDR 1 is clearly solvent-exposed, whereas the degree of exposure of CDR 1 residue 31 likely depends on the sequence and structure of the V_H CDR 3. With respect to the non–solvent-exposed CDR 3 residue 96, the four most closely juxtaposed amino acids are heavy chain residues 99 and 105 (V_H CDR 3), light chain residue 99 (Vκ CDR 3), and light chain residue 37 (Vκ FR2). Residue 96 in the CDR 3 region may thus affect the conformation of the antibody binding site in one of three ways: directly, indirectly through contacts with the CDR 3 of the light and heavy chain, or by internal interactions with the FR 2 core region. Introduction of a charged residue into this region of the CDR 3 domain may thus influence heavy and light chain pairing.

In germline form, the Humkv325 gene segment encodes a CRI that can be detected by the 17.109 mAb (52). As is typical for patients with RA, the 17.109 CRI could be detected on less than 1% of serum RF, synovial cell-derived RF, or synovial cell-derived total immunoglobulin from this patient (11). However, our random cDNA library analysis demonstrated that ~ 12.5% of the κ light chain transcripts were drawn from the Humkv325 germline gene segment. We presume the absence of 17.109 reactivity in the presence of the Humkv325 gene may be explained, at least in part, by somatic mutation in the CDR regions that define the epitope. We are currently in the process of expressing these transcripts to test this hypothesis. Our results provide a further caveat for studies that rely exclusively on use of idiotypic antibodies for analysis of the expressed antibody repertoire.

Several biologic questions of interest remain unanswered as a result of limitations of cDNA analysis. The antigen specificities of antibodies containing the described clonally related Vκ gene segments, including potential differences in reactivities of closely related Vκ gene segments (e.g., 28S1 and 37S4) are unknown, but expression of light chains in prokaryotic systems may provide useful insights in this regard. The identity of heavy chains paired with these light chains remains unknown. Single-cell PCR, which allows correlation of heavy chains with light chains, may eliminate this problem in future studies.

Antibodies containing light chains with 10S2-like CDR 3 regions were found only in this patient's synovium, but not in other tissues sampled, including the patient's PBLs, and spleen lymphocytes and PBLs from normal individuals. Although unaffected synovial tissue would theoretically provide a better control, normal synovial tissue does not contain B lineage cells. Analysis of synovial tissue obtained simultaneously from different joints in the same patient could potentially address the question of whether there are tissue-specific or local differences in immunoglobulin gene segment expression. Similar studies of additional patients should provide information regarding inter-individual variation.

In summary, we have provided proof of oligoclonal expansion of B cells expressing the Humkv325 gene segment in the involved synovium of a patient with longstanding RA. The high level of expression of at least one clone suggests that we may be able to identify the companion heavy chain sequence and thus gain insight into the antigen specificity of this antibody. The roles of somatic mutation, variation in the length of the light chain CDR 3 region, and N nucleotide addition in the normal light chain repertoire and the identity of the putative antigen(s) that may have elicited the B cell proliferative response remain major open questions that will be the focus of future investigations.

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References

1. Mellors, R. C., R. Heimer, J. Corcos, and L. Korngold. 1959. Cellular origin of rheumatoid factor. *J. Exp. Med.* 110:875–886.

2. Harris, Jr., E. D. 1990. Rheumatoid arthritis: Pathophysiology and implications for therapy. *N. Engl. J. Med.* 322:1277–1289.

3. Bankhurst, A. D., G. Husby, and R. C. Williams, Jr. 1976. Predominance of T cells in the lymphocytic infiltrates of synovial tissues in rheumatoid arthritis. *Arthritis Rheum.* 19:555–562.

4. Smiley, J. D., C. Sachs, and M. Ziff. 1968. In vitro synthesis of immunoglobulin by rheumatoid synovial membrane. *J. Clin. Invest.* 47:624–632.

5. Panush, R. S., N. E. Bianco, and P. H. Schur. 1971. Serum and synovial fluid IgG, IgA, and IgM antigammaglobulins in rheumatoid arthritis. *Arthritis Rheum.* 14:737–747.

6. Withrington, R. H., I. Teitsson, H. Valdimarsson, and M. H. Seifert. 1984. Prospective study of early rheumatoid arthritis. II. Association of rheumatoid factor isotypes with fluctuations in disease activity. *Ann. Rheum. Dis.* 43:679–685.

7. Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (Lond.).* 302:575–581.

8. Kabat, E. A., T. T. Wu, H. M. Perry, K. S. Gottesman, and C. Foeller. 1991. Sequences of Proteins of Immunological Interest. U. S. Department of Health and Human Services, Bethesda, MD. 2,597 pp.

9. Meindl, A., H.-G. Klobeck, R. Ohnheiser, and H. G. Zachau. 1990. The Vκ gene repertoire in the human germline. *Eur. J. Immunol.* 20:1855–1863.

10. Kelley, D. E., and R. P. Perry. 1986. Transcriptional and posttranscriptional control of immunoglobulin mRNA production during B lymphocyte development. *Nucleic Acids Res.* 14:5431–5447.

11. Lee, S. K., S. L. Bridges, Jr., W. J. Koopman, and H. W. Schroeder, Jr. 1992. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. *Arthritis Rheum.* 35:905–913.

12. Kipps, T. J., B. A. Robbins, and D. A. Carson. 1990. Uniform high frequency expression of autoantibody-associated crossreactive idiotypes in the primary B cell follicles of human fetal spleen. *J. Exp. Med.* 171:189–196.

13. Kipps, T. J., B. A. Robbins, P. Kuster, and D. A. Carson. 1988. Autoantibody-associated cross reactive idiotypes expressed at high frequency in chronic

lymphocytic leukemia relative to B-cell lymphomas of follicular center cell origin. *Blood.* 72:422–428.

14. Kipps, T. J., S. Fong, E. Tomhave, P. P. Chen, R. D. Goldfien, and D. A. Carson. 1987. High-frequency expression of a conserved kappa light-chain variable-region gene in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci., USA.* 84:2916–2920.

15. Harindranath, N., I. S. Goldfarb, H. Ikematsu, S. E. Burastero, R. L. Wilder, A. L. Notkins, and P. Casali. 1991. Complete sequence of the genes encoding the V_H and V_L light of low- and high-affinity monoclonal IgM and IgA1 rheumatoid factors produced by $CD5^+$ B cells from a rheumatoid arthritis patient. *Int. Immunol.* 3:865–875.

16. Chen, P. P., K. Albrandt, N. K. Orida, V. Radoux, E. Y. Chen, F.-T. Liu, and D. A. Carson. 1986. Genetic basis for the cross-reactive idiotypes on the light chains of human IgM anti-IgG autoantibodies. *Proc. Natl. Acad. Sci., USA.* 83:8318–8322.

17. Radoux, V., P. P. Chen, J. A. Sorge, and D. A. Carson. 1986. A conserved human germline Vκ gene directly encodes rheumatoid factor light chains. *J. Exp. Med.* 164:2119–2124.

18. Pons-Estel, B., F. Goni, A. Solomon, and B. Frangione. 1984. Sequence similarities among kappa IIIb chains of monoclonal human IgM kappa autoantibodies. *J. Exp. Med.* 160:893–904.

19. Dersimonian, H., K. P. W. J. McAdam, C. Mackworth-Young, and B. D. Stollar. 1989. The recurrent expression of variable region segments in human IgM anti-DNA autoantibodies. *J. Immunol.* 142:4027–4033.

20. Ledford, D. K., F. Goni, M. Pizzolato, E. C. Franklin, A. Solomon, and B. Frangione. 1983. Preferential association of kappa IIIb light chains with monoclonal human IgM kappa autoantibodies. *J. Immunol.* 131:1322–1325.

21. Suh, S. W., T. N. Bhat, M. A. Navia, G. H. Cohen, D. N. Rao, S. Rudikoff, and D. R. Davies. 1986. The galactan-binding immunoglobulin Fab J539: an X-ray diffraction study at 2.6 Å resolution. *Proteins.* 1:74–80.

22. Bernstein, F. C., T. F. Koetzle, G. J. B. Williams, E. F. Meyer, Jr., M. D. Brice, J. R. Rodgers, O. Kennard, T. Shimaouchi, and M. Tasumi. 1977. The Protein Data Bank: a computer-based archival file for macromolecular structures. *J. Mol. Biol.* 112:535–542.

23. Abola, E. E., F. C. Bernstein, S. H. Bryant, T. F. Koetzle, and J. Weng. 1987. Crystallographic Databases—Information Content, Software Systems, Scientific Applications. F. H. Allen, G. Bergerhoff, and R. Sievers, editors. Data Commission of the International Union of Crystallography, Bonn/Cambridge/Chester. 107–132.

24. English, D., and B. R. Andersen. 1974. Single-step separation of red blood cells, granulocytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. *J. Immunol. Meth.* 5:249–252.

25. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

26. Uematsu, Y. 1991. A novel and rapid cloning method for the T cell receptor variable region sequence. *Immunogenetics.* 34:174–178.

27. Larrick, J. W., L. Danielsson, C. A. Brenner, M. Abrahamson, K. E. Fry, and C. A. K. Borrebaeck. 1989. Rapid cloning of rearranged immunoglobulin genes from human hybridoma cells using mixed primers and the polymerase chain reaction. *Biochem. Biophys. Res. Commun.* 160:1250–1255.

28. Straubinger, B., E. Huber, W. Lorenz, E. Osterholzer, W. Pargent, M. Pech, H.-D. Pohlenz, F.-J. Zimmer, and H. G. Zachau. 1988. The human Vκ locus. Characterization of a duplicated region encoding 28 different immunoglobulin genes. *J. Mol. Biol.* 199:23–34.

29. Klobeck, H.-G., G. W. Bornkamm, G. Combriato, R. Mockikat, H.-D. Pohlenz, and H. G. Zachau. 1985. Subgroup IV of human immunoglobulin light chains is encoded by a single germline gene. *Nucleic Acids Res.* 13:6515–6529.

30. Klobeck, H.-G., A. Meindl, G. Combriato, A. Solomon, and H. G. Zachau. 1985. Human immunoglobulin kappa light chain genes of subgroups II and III. *Nucleic Acids Res.* 13:6499–6513.

31. Jukes, T. H., and J. L. King. 1979. Evolutionary nucleotide replacements in DNA. *Nature (Lond.).* 281:605–606.

32. Shlomchik, M. J., A. Marshak-Rothstein, C. B. Wolfowicz, T. L. Rothstein, and M. G. Weigert. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature (Lond.).* 328:805–811.

33. Shlomchik, M. J., A. H. Aucoin, D. S. Pisetsky, and M. G. Weigert. 1987. Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc. Natl. Acad. Sci., USA.* 84:9150–9154.

34. Shlomchik, M. J., D. A. Nemazee, V. L. Sato, J. van Snick, D. A. Carson, and M. G. Weigert. 1986. Variable region sequences of murine IgM anti-IgG monoclonal autoantibodies (rheumatoid factors). A structural explanation for the high frequency of IgM anti-IgG B cells. *J. Exp. Med.* 164:407–427.

35. Jacob, J., G. Kelsoe, K. Rajewsky, and U. Weiss. 1991. Intraclonal generation of antibody mutants in germinal centres. *Nature (Lond.).* 354:389–392.

36. Tillman, D. M., N.-T. Jou, R. J. Hill, and T. N. Marion. 1992. Both IgM and IgG anti-DNA antibodies are the products of clonally selective B cell stimulation in (NZB × NZW)F_1 mice. *J. Exp. Med.* 176:761–779.

37. Marion, T. N., D. M. Tillman, N.-T. Jou, and R. J. Hill. 1992. Selection of immunoglobulin variable regions in autoimmunity to DNA. *Immunol. Rev.* 128:1–27.

38. Koopman, W. J., R. E. Schrohenloher, S. S. Crago, D. M. Spalding, and J. Mestecky. 1985. IgA rheumatoid factor synthesis by dissociated synovial cells. *Arthritis Rheum.* 28:1219–1227.

39. Sanz, I. 1991. Multiple mechanisms participate in the generation of diversity of human H chain CDR 3 regions. *J. Immunol.* 147:1720–1729.

40. Heller, M., J. D. Owens, J. R. Mushinski, and S. Rudikoff. 1987. Amino acids at the site of Vκ–Jκ recombination not encoded by germline sequences. *J. Exp. Med.* 166:637–646.

41. Sanz, I., and J. D. Capra. 1987. Vκ and Jκ gene segments of A/J Ars-A antibodies: somatic recombination generates the essential arginine at the junction of the variable and joining regions. *Proc. Natl. Acad. Sci., USA.* 84:1085–1089.

42. Scott, M. G., J. J. Tarrand, D. L. Crimmins, D. W. McCourt, N. R. Siegel, C. E. Smith, and M. H. Nahm. 1989. Clonal characterization of the human IgG antibody repertoire to *Haemophilus influenzae* type b polysaccharide. II. IgG antibodies contain V_H genes from a single V_H family and V_L genes from at least four V_L families. *J. Immunol.* 143:293–298.

43. Marks, J. D., M. Tristem, A. Karpas, and G. Winter. 1991. Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable genes and design of family-specific oligonucleotide probes. *Eur. J. Immunol.* 21:985–991.

44. Victor, K. D., I. Randen, K. Thompson, O. Førre, J. B. Natvig, S. M. Fu, and J. D. Capra. 1991. Rheumatoid factors isolated from patients with autoimmune disorders are derived from germline genes distinct from those encoding the Wa, Po, and Bla cross reactive idiotypes. *J. Clin. Invest.* 87:1603–1613.

45. Pratt, L. F., L. Rassenti, J. Larrick, B. Robbins, P. M. Banks, and T. J. Kipps. 1989. Ig V region gene expression in small lymphocytic lymphoma with little or no somatic hypermutation. *J. Immunol.* 143:699–705.

46. Alt, F. W., and D. Baltimore. 1982. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D–J_H fusions. *Proc. Natl. Acad. Sci., USA.* 79:4118–4122.

47. Desiderio, S. V., G. D. Yancopoulos, M. Paskind, E. Thomas, M. A. Boss, N. Landau, F. W. Alt, and D. Baltimore. 1984. Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. *Nature (Lond.).* 311:752–755.

48. Landau, N. R., D. G. Schatz, M. Rosa, and D. Baltimore. 1987. Increased frequency of N-region insertion in a murine pre-B cell line infected with a terminal deoxynucleotidyl transferase retroviral expression vector. *Mol. Cell Biol.* 7:3237–3243.

49. Klobeck, H. G., G. Combriato, and H. G. Zachau. 1987. N segment and region-directed somatic hypermutation in kappa gene of a t(2;8) chromosomal translocation. *Nucleic Acids Res.* 15:4877–4888.

50. Goodhart, M., C. Babinet, G. Lutfalla, S. Kallenbach, P. Cavelier, and F. Rougeon. 1989. Immunoglobulin kappa light chain gene promoter and enhancer are not responsible for B-cell restricted gene rearrangement. *Nucleic Acids Res.* 17:7403–7415.

51. Martin, T., G. Blaison, H. Levallois, and J. L. Pasquali. 1992. Molecular analysis of the VκIII–Jκ junctional diversity of polyclonal rheumatoid factors during rheumatoid arthritis frequently reveals N addition. *Eur. J. Immunol.* 22:1773–1779.

52. Jirik, R. F., J. Sorge, S. Fong, J. G. Heitzmann, J. G. Curd, P. P. Chen, R. Goldfien, and D. A. Carson. 1986. Cloning and sequence determination of a human rheumatoid factor light-chain gene. *Proc. Natl. Acad. Sci., USA.* 83:2195–2199.