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Frequent N Addition and Clonal Relatedness among Immunoglobulin Lambda Light Chains Expressed in Rheumatoid Arthritis Synovia and PBL, and the Influence of Vλ Gene Segment Utilization on CDR3 Length

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Abstract

Background: In rheumatoid arthritis (RA), B-lineage cells in the synovial membrane secrete large amounts of immunoglobulin that contribute to tissue destruction. The CDR3 of an immunoglobulin light chain is formed by rearrangements of V_L and J_L gene segments. Addition of non-germline-encoded (N) nucleotides at V(D)J joins by the enzyme terminal deoxynucleotidyl transferase (TdT) enhances antibody diversity. TdT was previously thought to be active in B cells only during heavy chain rearrangement, but we and others reported unexpectedly high levels of N addition in kappa light chains. We also found clonally related kappa chains bearing unusually long CDR3 intervals in RA synovium, suggesting oligoclonal expansion of a set of atypical B lymphocytes. In this study, we analyzed lambda light chain expression to determine if N addition occurs throughout immunoglobulin gene rearrangement and to compare CDR3 lengths of lambda and kappa light chains in RA patients and normal individuals.

Materials and Methods: Reverse transcription–polymerase chain reaction (RT-PCR) amplification of VλIII transcripts was performed on RA synovia and peripheral blood lymphocytes (PBL) and normal PBL for which kappa repertoires were previously analyzed. Representative λ^+ PCR products were cloned and sequenced.

Results: Analysis of 161 cDNA clones revealed that N addition occurs in lambda light chains of RA patients and normal controls. The lambda light chain repertoires in RA were enriched for long CDR3 intervals. In both RA and controls, CDR3 lengths were strongly influenced by which Vλ gene segment was present in the rearrangement. Five sets of clonally related sequences were found in RA synovia and PBL; one set was found in normal PBL.

Conclusions: In humans, unlike mice, N addition enhances antibody diversity at all stages of immunoglobulin assembly, and the structural diversity of lambda CDR3 intervals is greater than that of kappa light chains. Clonally related Vλ gene segments in RA support an antigen-driven B-cell response.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic illness characterized by inflammation and hyperplasia of the synovial membrane of affected joints (1–3). Infiltration of the synovium by B lymphocytes and plasma cells is a hallmark of the disease and often contributes to joint destruction (4,5). Antibodies are heterodimeric proteins composed of two immunoglobulin heavy (H) chains and two light (L) chains, each of which has a variable (V) domain for antigen recognition. In order to recognize the virtually limitless number of antigens in the environment, multiple mechanisms to generate antibody diversity have evolved. In humans, these include the use of many differ-

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ent gene segments to form antibodies (6), differential pairing of different heavy and light chains, somatic hypermutation, and the insertion of non-germline-encoded (N) nucleotides at the sites of gene segment rearrangement (reviewed in refs. 7–9).

Variable heavy-chain domains are encoded by variable (V_H), diversity (D_H), and joining (J_H) gene segments, which undergo sequential somatic rearrangements to become juxtaposed in the genomic DNA of the B cell (8). After heavy chain rearrangement, the kappa locus undergoes rearrangements of V and J gene segments to generate light chain variable domains. If kappa light chain rearrangement fails on both alleles, lambda light chain V-J rearrangements occur. In humans, about 40% of expressed antibodies contain lambda light chains (9). V_H and V_L gene segments encode three framework regions (FR1, FR2, and FR3), which are separated by two highly variable domains, termed complementarity determining regions (CDRs). The regions of the V_H-D_H-J_H and V_L-J_L joins define the heavy and light chain CDR3 intervals, respectively, which are at the center of the antigen-binding site (10) and are usually directly involved in antigen binding.

Non-germline-encoded (N) nucleotides are added at the $D_H \rightarrow J_H$ and $V_H \rightarrow D_H$ junctions of immunoglobulin heavy chains by the enzyme deoxynucleotidyl transferase (TdT). Until recently, expression of TdT was thought to be restricted to the pro-B-cell stage of B-cell development, when most heavy-chain rearrangement occurs (11). However, we and others have shown that N-region addition can occur in Vκ-Jκ joins of B cells of normal individuals (12–16) and patients with RA (17,18). As was the case with kappa light chains until recently, N-region addition is not thought to occur in V-J joins of lambda light chains (19), but this issue has not been formally addressed. Sequencing of the human lambda locus (20,21) has made such analysis feasible by allowing assignment of V and J gene segment progenitors.

Although N-region addition can occur in kappa light chains, the length of the CDR3 intervals is highly regulated in humans, with >95% of sequenced kappa chain CDR3s from normal peripheral B cells having either 9 or 10 amino acids (10). We have shown that in some RA patients, the kappa light repertoire is enriched for unusually long CDR3 intervals (16,22). This finding may be due to differences in regulation of gene rearrangement, for example, prolonged expression of TdT, or to antigenic selection of B lymphocytes expressing unusual antibodies.

The goals of the present study were to determine the degree of N-region addition and CDR3-length heterogeneity in lambda light chains expressed in RA patients and normal controls. We focused on expression of the largest Vλ family, VλIII (21), which is frequently used in autoantibodies from patients with RA (23–26). To avoid potential bias in the results by selecting for antibodies that express a particular reactivity, we used a reverse transcription–polymerase chain reaction (RT-PCR) approach on unsorted cells from synovial tissue and peripheral blood lymphocytes (PBL) of two patients with RA and PBL of three normal controls. Vκ-Jκ joins expressed in these same samples have been extensively characterized, which allowed comparison of findings in the kappa and lambda repertoires.

We report analysis of a total of 194 Vλ sequences—161 cDNA sequences and 33 unrearranged genomic DNA sequences (derived from 6 germline VλIII gene segments). Extensive N-region addition was found in the Vλ-Jλ joins of both RA patients and normal controls. Surprisingly, two of three normal individuals exhibited more N-region addition in lambda light chains than in kappa light chains. The lambda repertoires of the RA patients were enriched for unusually long CDR3 intervals, similar to the pattern previously reported for their kappa repertoires. The CDR3 lengths of lambda light chains in both RA and normal individuals were affected by both N-region addition and by the length of the Vλ gene segment utilized in the rearrangement. The enrichment for uncommon CDR3 structures and evidence of oligoclonal B cell expansion in RA provides further support for the hypothesis that changes in the repertoire reflect selection by an unknown antigen or sets of antigens.

Materials and Methods

Patient Characteristics and Isolation of Synovial Cells and Peripheral Blood Mononuclear Cells

Clinical characterization of the RA patients (BC and AS) and normal controls used in this study and the methods used to process the synovial tissue and peripheral blood have been reported

previously (16,17). Patients BC (62, WF) and AS (42, BF), as well as normal controls LB (36, WM), LK (23, WF), and IT (62, WF) have been subjects of in-depth analysis of V gene segment utilization, N-region addition, and CDR3-length variability of the kappa light chain repertoire (16,17,27). Synovial tissue and PBL were obtained from AS in close temporal proximity. In this study, PBL from patient BC were obtained several years after isolation of synovial tissue because the supply of RNA from PBL obtained at the time of surgery had been exhausted.

Generation of cDNA and PCR Amplification of Vλ-containing Transcripts

Total RNA was isolated from each sample using the guanidinium isothiocyanate technique (28) or using Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH). Oligo d(T)–primed first-strand cDNA was generated from total RNA as previously described (16). PCR amplifications were performed on 2-μl aliquots of first-strand cDNA, using *Taq* DNA polymerase as previously described (16). PCR conditions were as follows: 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 4 min, with a final extension at 72°C for 10 min. A low annealing temperature was used to decrease the likelihood that mutated sequences would be missed.

The VλIII family contains three subfamilies defined serologically and by sequence analysis (21,29–31). Thus, three separate PCR amplifications were performed on each tissue sample to enrich for sequences containing VλIIIa-, VλIIIb-, and VλIIIc-derived gene segments. The three sense primers used were derived from germline leader sequences and were each used in conjunction with an antisense consensus Cλ primer, LB-69. Leader sequences used were LB-75 (VλIIIa), LB-76 (VλIIIb), and LB-77 (VλIIIc) (Fig. 1). To control for possible contamination, mock PCR reaction mixtures lacking template or containing products of the first-strand cDNA reaction without reverse transcriptase were prepared. None of the controls contained amplified product as assessed by ethidium-stained agarose gel electrophoresis or Southern blot analysis using a ^{32}P-labeled internal Cλ oligonucleotide, LB-70 (Fig. 1).

Isolation of Genomic DNA from non-B Cells and Amplification of Germline Vλ Gene Segments

In an attempt to allow definitive assignment of cDNA sequences to germline progenitor gene segments, we sequenced unrearranged VλIII gene segments from genomic DNA of T lymphocytes from two individuals (RA patient BC and normal control LB). PBL were obtained by standard Ficoll-Hypaque gradient centrifugation (32). T lymphocytes were isolated from PBL by sheep red blood cell (SRBC) rosetting (33). Briefly, 1 ml of 25% 2-aminoethylisothiouronium bromide (AET)-SRBC was added per 10^8 PBL. The PBLs were resuspended in ~10 ml 5% fetal calf serum (FCS)/10^8 PBL. The PBL-SRBC solution was distributed evenly into sterile round-bottom 15-ml tubes and centrifuged for 5 min. The tubes were placed on ice for 30 min, then the pellets were resuspended in ice cold 5% FCS. Aliquots of 40 × 10^6 cells were pipetted onto Ficoll-Hypaque and centrifuged at 1800 rpm at 4°C for 30 min. The interface, which contained the B lymphocytes, was removed. The T cells in the pellet were then washed three times and genomic DNA was extracted, purified by phenol extraction, and precipitated in ethanol using standard techniques (34).

PCR amplifications were performed on 2-μl aliquots of genomic DNA. PCR conditions were as follows: preheat to 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 1 min, with a final extension at 72°C for 7 min. Two separate PCR amplifications were performed on each genomic DNA, one to amplify VλIIIa and VλIIIc gene segments (using upstream primer LB-123 and downstream primer LB-125), and one to amplify VλIIIb gene segments (using upstream primer LB-124 and downstream primer LB-125) (Fig. 1). Primer LB-123 was derived from the intronic sequences of the VλIIIa gene segments IGLV3S2 (35) and humlv318 (29), and the VλIIIc gene segment III.1 (30). Primer LB-124 was derived from intron sequences from the VλIIIb gene segments hsiggll150 (26) and hsiggll295 (26). Primer LB-125 was derived from the heptamer, 23 base pair spacer, and nonamer sequences of the recombination signal sequences (RSS) of the IGLV3S2, humlv318, and III.1 gene segments (Fig. 1C). PCR products were probed with an internal probe, LB-126, derived from a portion of the

A

Primer	Gene	Region	Orientation	Sequence (5'→3')
LB-75	VλIIIa	Leader	Sense	TCCGAATTCTCCTCTCTCACTGCACAG
LB-76	VλIIIb	Leader	Sense	TCCGAATTCTCTGCACAGTCTCTGAGGCC
LB-77	VλIIIc	Leader	Sense	CCCTGAATTCCTCGGCGTCCTTGCTTACTGCA
LB-69	Cλ	Constant	Antisense	GGGAATTCGCTCCCGGGTAGAAGTCACT
LB-70	Cλ	Internal	Antisense	GGGAATTCTTG(GA)CTTGGAGCTCCTCAGAGGAGGG
LB-123	VλIIIa/c	Intron	Sense	TCCAGC(CG)TG(GT)CC(CT)TGA(CT)TCTGAGCTCAGGA
LB-124	VλIIIb	Intron	Sense	GTGCT(GT)(CT)(CT)CCCAGGCCCTGCTCCAGGC
LB-125	VλIII	RSS	Antisense	(GT)GTTT(CT)TGTCTCACTTCC(GT)CATCTGCCTGTGT(CT)AC(CT)GTG
LB-126	VλIII	FR2	Sense	CAGCAGAAGCCAGGCCAG

B

Leader Sequences

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VλIIIa lv318      ATGGCCTGGACCGTTCTCCTCCTCGGCCTCCTCTCTCACTGCACAGGTGATCCCCCC
       iglv3s2    ............................................................
       LB-75                              ...GAATTC..................
VλIIIb hsiggll150 ..........T.CC...A..T...CC.......A...T.........CTC.GAGG..
       hsiggll295 ............CC......G...CC.......A..TT........TCTC.GAGG..
       LB-76                                      ...GAATTCT........TCTC.GAGG..
VλIIIc III.1      .....A....T.CC....T........G....TG..T............C.G....T
       LB-77                   .CC.GAATTC......G....TG..T......
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C

Recombination Signal Sequences

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        Heptamer  |←          Spacer          →| Nonamer
3S2     CACGGTA   ACACAGGCAGATGAGGAAGTGAG       ACAAAAACA
lv318   ......G   ........................       .........
III.1   ...A..G   .............C.........       ...G....C
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Fig. 1. (A) Sequences of oligonucleotides used as primers and probes. Parentheses indicate sites of degeneracy. RSS, recombination signal sequence; FR2, framework 2. (B) Comparison of VλIIIa, b, and c leader sequences and primers used for RT-PCR to the germline humlv318 sequence. Dots indicate nucleotide sequence homology. Underlined sequences indicate *Eco*RI restriction sites. (C) Sequences of RSS of gene segments IGLV3S2, lv318, and III.1.

framework 2 domain that is highly conserved among VλIII gene segments.

Cloning and Sequencing of PCR Products

Aliquots of PCR products were subcloned using the TA cloning kit (Invitrogen, San Diego, CA). Plasmids were transformed into INVαF$^-$ *E. coli* by electroporation using a Bio-Rad Gene Pulser (Hercules, CA). For colonies that hybridized to the Cλ probe, plasmid DNA was obtained and sequenced by the dideoxy chain termination procedure (36) either manually or using an automated sequencer (Applied Biosystems, Inc., Model AB373).

Sequence Analysis

The FR and CDR domains of each of the sequences were compared individually to corresponding domains of published human Vλ sequences using the computer program SAW (Sequence Analysis Workshop) (37). Sequences were assigned to germline gene segments according to highest degree of nucleotide sequence homology. Levels of somatic

Table 1A. Germline derivation of Vλ gene segments in cDNA clones from patients with rheumatoid arthritis and normal controls

	Vλ Family	VλI		VλIIIa			VλIIIb				VλIIIc	VλIV			VλV				VλVI	
	Gene Segment	lv1042	DPL6	3S2	lv318	2-14	150	3a	3p	2-19	III.1	8A1	lv801	5-4	5a	5c1	5c2	4-2	6S1	Total
RA	AS Synovium	1	1	5	3	0	10	0	1	1	5	1	1	0	0	1	0	0	1	31
	AS PBL	3	0	1	4	0	8	0	0	0	6	0	0	0	0	0	0	0	0	22
	BC Synovium	1	0	4	0	1	4	0	4	0	2	1	0	0	0	0	0	0	4	21
	BC PBL	0	0	1	0	2	1	0	5	0	6	0	0	0	1	0	2	1	1	20
Normal	LB PBL	1	0	0	0	3	6	0	0	0	3	1	0	1	0	1	0	0	2	18
	IT PBL	0	0	1	0	5	11	1	0	0	7	1	0	0	1	0	1	0	1	29
	LK PBL	2	0	0	0	0	6	0	1	1	7	0	0	1	0	0	2	0	0	20
	Total	8	1	12	7	11	46	1	11	2	36	4	1	2	2	2	5	1	9	161

Germline gene segments in Table 1A were reported as follows: lv1042 (42) , DPL6 (43), 3S2 (35), lv318 (29), 2-14, 2-19, 4-2, and 5-4 (21), hsiggll150 (20, 26), 3a, 3p, 5a, 5c1, and 5c2 (20), III.1 (20, 30), 8A1 (44), lv801 (45), and 6S1 (46).

hypermutation were assessed by comparing the FR1 through the FR3 domains (codons 1–88 according to ref. 10) of each complete transcript to the appropriate germline sequence and calculating mean divergence for each sample. Truncated clones were not included in the somatic mutation analysis.

P nucleotides are nucleotides that are palindromic to terminal nucleotides of coding sequences (38). They are inserted adjacent to the corresponding coding sequences at the junction of rearranged gene segments and are thought to be a consequence of the resolution of hairpin structures that occur during V(D)J recombination (39). In this study, nucleotide mismatches at the 3′-end of the Vλ gene segment or the 5′-end of the Jλ gene segment that did not appear to be palindromic to the V or J were assumed to represent N-region addition rather than somatic mutation. In order to allow valid comparisons between lambda and kappa chains expressed in the same samples, sequences of previously reported Vκ-Jκ joins (16) were reanalyzed to look for the presence of P nucleotides.

Statistical Analysis

Differences in the amount of somatic mutation, N-region addition, and CDR3-length heterogeneity between patients with RA and normal individuals were analyzed using the Chi-square test, Fisher exact test (two-tailed), or Student *t*-test, as appropriate. Three-way comparisons were performed using analysis of variance (ANOVA).

Results

Germline Vλ Gene Segment Derivation of cDNA Sequences

In humans, there are three separate clusters of Vλ gene segments on chromosome 22q11.2 (40). There are a total of ~69 Vλ gene segments, including ~36 functional Vλ gene segments and ~33 pseudogenes, which are grouped into 10 families on the basis of nucleotide sequence homology (20,21). Two gene segments that are not part of the major locus, so-called orphon sequences, have been reported on chromosome 8q11.2 (41). Allelic polymorphism of the human lambda locus appears to be limited (20). As mentioned above, the VλIII family is the largest Vλ family, composed of 10 functional members and 13 pseudogenes, which are subdivided into three subgroups (a, b, and c) (21,29–31).

The Vλ gene segments of 161 cDNA clones were assignable to known germline gene segments (Table 1A). Of 10 VλIII germline gene segments with open reading frames (20,21), 123 of 126 (97.6%) VλIII transcripts were derived from six gene segments: VλIIIa gene segments IGLV3S2 (35) (12 clones, 7.5% of the total), 2-14 (21) (11 clones, 6.9%), and humlv318 (29) (7 clones, 4.4%); VλIIIb gene segments hsiggll50 (3m) (20,26) (46 clones, 28.8%) and 3p (20) (11 clones, 6.9%); and VλIIIc gene segment III.1 (3r) (20,30) (35 clones, 21.9%). This pattern of VλIII gene utilization is similar to that found by other investigators, who assessed the expression of Vλ

gene segments by screening cDNA libraries generated from normal PBL (47). In that study, the most commonly expressed VλIII gene segments were 3h (which includes closely related gene segments IGLV3S2, humlv318, and 2-14; ref. 20), 3r (III.1), 3p, and 3m (hsiggll150), with less frequent representation of 3e, 31, 3a, and 3j. In the present study, because of cross-reactivity of the PCR primers, members of the VλI (9 clones, 5.6%), VλIV (8 clones, 5.0%), VλV (10 clones, 6.3%), and VλVI (9 clones, 5.6%) families were also represented.

Several clones were found to have deletions or insertions in the V gene segment (Figs. 2 and 3). ASSynL191 had a three-nucleotide deletion in CDR1 and LKPBLL68 contained a three-nucleotide insertion in CDR1. LKPBLL10 had one nucleotide missing from FR2 and a one–base pair insertion in the CDR3 interval. ITPBLL75 contained a ten–base pair deletion at the FR3-CDR3 junction. Single base pair insertions in CDR3 were seen in clones LBPBLL4, ITPBLL4, and LKPBLL53. Overall, there were 5 out-of-frame cDNA sequences, all of which were isolated from normal PBL.

Derivation of Unrearranged Vλ Gene Segments Amplified from T Lymphocytes

Of 33 clones from two different individuals (RA patient BC and normal control LB), six different gene segments [3i, hsiggll150 (3m), 3a, 3p, III.1 (3r), and 3e] were represented (Table 1B). Thirty-one of 33 (94%) of the clones were 100% identical to known germline gene segments. Clone LBLG9 was found to have two nucleotide differences from the reported 3p sequence: GGG instead of GAG at codon 50 in CDR2, resulting in a glycine residue instead of glutamine, and ATG instead of GTG at codon 80 in FR3, resulting in a methionine residue rather than a valine. Clone LBLB14 had three nucleotide differences from the reported III.1 sequence: ACG instead of ACA at codon 18 in FR1, which does not change the encoded threonine residue, GCA instead of ACA at position 70 in FR3, resulting in alanine instead of threonine, and GTT (Val) instead of GCT (Ala) at codon 80 in FR3. None of the clones reported here appeared to be derived from these two variant sequences. One or more of these five sequence changes may represent PCR artifact induced by *Taq* DNA polymerase (see below).

Possible Allelic Variants of Vλ Gene Segments

Among the cDNA sequences, we found multiple sets of clones that had shared differences in the Vλ gene segments (Table 2) but were definitely not clonally related, as they utilized different Jλ gene segments or had discordant N-region addition and/or CDR3 lengths (Fig. 3). This finding suggests the presence of novel allelic variants of germline gene segments IGLV3S2, III.1, hsiggll150, and iglv6S1, or the presence of novel gene segments closely related to those germline genes. In the sequence analysis of unrearranged gene segments (see above), we did not isolate germline sequences containing the variant codons shown in Table 2. Possible explanations for this discrepancy are the small number of genomic sequences we analyzed or sequence mismatches between our PCR primers and intronic sequences of allelic variants.

Fig. 2. Deduced amino acid sequences of Vλ cDNA clones from RA synovia, RA PBL, and normal PBL. Dots indicate sequence homology; single-letter amino acid abbreviations are used, X, stop codon in deduced amino acid sequence. *, termination of cDNA transcript. ‡, Clone BCSynL32 contains FR1 and CDR1 domains from Vλ gene segment 6S1 and FR2 through CDR3 domains from Vλ gene segment DPL8. §, Clones containing insertions or deletions: ASSynL191 has a three-nucleotide deletion in CDR1, denoted by dash; ASPBLL54 has a two–base pair deletion in FR1; ITPBLL4 has a one-nucleotide insertion in the CDR3 interval; ITPBLL75 contains a 10-nucleotide deletion at the FR3-CDR3 junction; LBPBLL4 has a one-nucleotide insertion in CDR3; LKPBLL10 contains a one-nucleotide deletion in FR2 and a one-nucleotide insertion (or a two nucleotide deletion) in CDR3; LKPBLL53 has a one-nucleotide insertion (or a two nucleotide deletion) in the CDR3 interval; LKPBLL68 has a three-nucleotide (one codon) insertion in CDR1. ‖, Clone BCSynL38 contains a stop codon in FR2. Genbank accession numbers for nucleotide sequences are as follows: AS Synovium: AF060120-AF060150; AS PBL: AF058057-AF058078; BC Synovium: AF063714-AF063734; BC PBL: AF063694-AF063713; IT PBL: AF063735-AF063762; LB PBL: AF063765-AF063782; LK PBL: AF064494-AF064513.

Rheumatoid Arthritis Synovia

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VλIII      |<-      FR 1        ->|<- CDR 1 ->|<-     FR 2  ->|<CDR2->|<-           FR3              ->|<-    CDR 3    ->|<-  FR4 ->|
IGLV3S2    SYVLTQPPSVSVAPGKTARITC GGNNIGSKSVH WYQQKPGQAPVLVIY YDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
ASSynL182  ...V..........Q..T... ..DDFEFLG.. ....R.....L...H S...... ................T........... ....IT..P      VI FGGGTKLTVL
ASSynL189  ....P.........Q..S... ...DF.LYG.. ...K........... C...... ............................... ...E.....      VV FGGGTKLTVL
ASSynL220  .................T... E.I..A..... ............... ...... ................R............ ....TG.A.      YV FGTGTKVTAL
ASSynL232  .........L.....Q..T... R.DD..I.... ..R.......A.... ..FH... ..............A...TS........... ...VRD..Q      RL FGGGTKLTVL
ASSynL234  ..................... ...D....P.. ............... ..N.... .........K.................S. ....NN...      WV FGGGTKLTVL
BCSynL4    ...........A...T..T... .....ET.... ............... .N..... ............H..............G... ....NTA..      YV FGGGTKVTVL
BCSynL21   .................T... ......R.... ......A........ S.R.... .........T..D.......T........F.. ....TRT..      YV FGTGTSLTVL
BCSynL22   .................T... ......R.... ......A........ S.R.... .........T..D.......T........F.. ....TRT..      YV FGTGTSLTVL
BCSynL24   ..................... ........... ............... ...... ............................... ........      YV FGTGTKVTVL
lv318      SYVLTQPPSVSVAPGKTARITC GGNNIGSKSVH WYQQKPGQAPVLVVY DDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
ASSynL186  ...V................. ...D......Q ............... ..T.... ...................N........E... ........P      YV FGSGTMVTVL
ASSynL221  .........E........... ..........Y ....R.......... ...... .............V................. ....NKG        V FGTGTKVTVV
ASSynL236  ..................... ......D.G.. ............... ...... .........K.R................... ....T..A.      YV FGTGTKVTAL
2-14       SYVLTQPPSVSVAPGQTARITC GGNNIGSKSVH WYQQKPGQAPVLVVY DDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
BCSynL36   ......S.A........... ........... ..H............ ..D.... ...........H.........V........ ...EI....      WV FGGGTKLTVL
hsiqll150  SYELTQPPSVSVSPGQTARITC SGDALPKQYAY WYQQKPGQAPVLVIY KDSERPS GIPERFSGSSSGTTVTLTISGVQAEDEADYYC QSADSSGTY
ASSynL184  ............Y...S.T... ......V.... .....A.....T..F E...... .........A..........E........... ....RL...      QV FGGGTHLTVL
ASSynL202     *.....C............ ...GF.S.FG. ..........I.L.. R.DQ... .........I.........R............ ....N..P.     YVA FGGGTRLTVL
ASSynL204  .........A........... ........... ....M......... T...... ............................... ....R...       WV FGGGTKLTVL
ASSynL209  ..................... .....SD.F.F ...........Q.M. LNTK... .......................SG....... .....D.S.      QV FGGGTKLTVL
ASSynL210  .FD................... ..ET..N..TF ...K...R...Q..Q ..T.... .........T...IA....T..H.D....... ....ITN.       WV FGGGTKLTVL
ASSynL211  ..................... ......D...F ...........Q... ..T.... ......................G....... ....RD.S.      QV FGGGTKLTVL
ASSynL214  .H................... ...G....... ...H.A.....R... E...... .............A.....EI.T........ E.E....      FWV FGGGTKVTVL
ASSynL215  ..................... ........... ..H.........L.. ..T.... ............................H. ..TG...P.      VV FGGGTRLTVL
ASSynL216  .ND...A.............. .....T...V. ...K.....L..... E.T.... ........AT.........T........... ..V..R.       KVD FGGGTKLTVL
ASSynL217  ..................... .A.......G. ............... ...... ............................R ........       PL FGGGTKLTVL
BCSynL17   ..................... ........... ....R......RI.. ...... ............................V.H. ....I...S      VI FGGGTKLTVL
BCSynL18   ..................... ...T..R..S. ..........I.L.. ...... .........T...................... ....NN.G.     QRI FGGGTKLTVL
BCSynL47   .S.........M......T... ...P.SL.F.. ...K......L..MS ..D.... ..S..L...R..A..N.I..E.......E... ....TR..       IV FGGGTKLTVL
BCSynL51   .........M..........N. .R..PLRA.F. .....A.....MI.F .NIG... .....I.A.....NS....NN........F.. ..T.TT.      NLL FGGGTKLTVL
3p         SYELTQPPSVSVSPGQTARITC SGDALPKKYAY WYQQKSGQAPVLVIY EDSKRPS GIPERFSGSSSGTMATLTISGAQVEDEADYYC YSTDSSGNHS
ASSynL136  ..................... .......Q... ............... ...... ...D......A........T............ .....GD..       RV FGGGTKLTVL
BCSynL16   ..................... ......R.... ...R..S.....L.. ..N.... EV....I..T...T................. .......     ELVV FGGGTRLTVL
BCSynL46   ..................... ........... ..R.R......Q... ..N.... .....L....................G.... .......     DQRV FGGGTKLTVL
BCSynL52   ..................... ......R.... ...R..S.....L.. ..N.... E.....I..T...T................. .......     ELVV FGGGTRLTVL
BCSynL53   ..................... ......R.... ...R..S.....L.. ..N.... E.....I..T...T................. .......     ELVV FGGGTRLTVL
2-19       SYELTQPSSVSVSPGQTARITC SGDVLAKKYAR WFQQKPGQAPVLVIY KDSERPS GIPERFSGSSSGTTVTLTISGAQVEDEADYYC YSAADNNL
ASSynL201  ..................... ..H........ ............... ...... ............................... .......       LGV FGGGTKLTVL
III.1      SYELTQPPSVSVSPGQTASITC SGDKLGDKYAC WYQQKPGQSPVLVIY QDSKRPS GIPERFSGSNSGNTATLTISGTQAMDEADYYC QAWDSSTA
ASSynL25   ...V.............N.P. ......N.AV. .....S....I..LF Y.TR... .......................S........ .T..N. SPWDNETAAV FGGGTKLTVR
ASSynL226  ..................... ..E.W.....S ............... ......L .........K..K................... .....        RKIV FGTGTKVTVL
ASSynL227  .F.V............K.N... ..EN.DE..V. ....R......M... E.GA... E............I..........I...E... ....        TNMGV FGGGTKLTVL
ASSynL230                          *.....V. .....S.......LF ..TR... ............................... .......       VV FGGGTKLTVL
ASSynL235  ..................... ..........S ............C .......................................... ........       V FGTGTKVTVL
BCSynL56   ..T...SS............. .....E..NTY .F........MM... .ND.... .........I..D..........SV....... .V....       IVV FGGGTKLTVL
BCSynL65   ..................... ........... ............... ...... ............................... .....G.       GV FGTGTKVTVL

VλI        |<-      FR 1        ->|<-   CDR 1  ->|<-     FR 2  ->|<CDR2->|<-           FR3              ->|<-    CDR 3    ->|<-  FR4 ->|
humlv1042  QSVLTQPPSVSGAPGQRVTISC TGSSSNIGAGYDVH WYQQLPGTAPKLLIY GNSNRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC QSYDSSLSGS
ASSynL188  ...............HNI.T.. ...R.....VF..N ....V.......... ..Y.... .......................D..G.... ....I.....      GV FGGGTKLTVL
BCSynL32 ‡ NFM....H...ES..KT..... .R..G..ASHFVQ  ............... ...... ............................... ........     WNYV FGTGTKVTVL
DPL6       QSVVTQPPSVSGAPGQRVTISC TGSSSNIGAGYDVH WYQQLPGTAPKLLIY GNSNRPS GVPDRFSGSKSGASASLAITGLQAEDEADYYC QSYDSSLSGS
ASSynL191 § ...L................. .........-H... ............... ..T.... .I............................. ........D.       V FGGGTKVTVL
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VλIV        |<-      FR 1        ->|<-  CDR 1  ->|<-     FR 2  ->|<- CDR2  ->|<-           FR3              ->|<-  CDR 3  ->|<-  FR4 ->|
8A1         QLVLTQSPSASASLGASVKLTC  TLSSGHSSYAIA WHQQQPEKGPRYLMK LNSDGSHSKGD GIPDRFSGSSSGAERYLTISSLQSEDEADYYC QTWGTGIH
ASSynL59    ................N...   .......D.T.. ....L.QR....... VK.....N... ............................... ......S.   WV FGGGTTLTVL
BCSynL37    ..EV..........D...F..   ....E.IT.... ....R.GV.L....T VTG....NL.H .............D......RV.......... .....     YVI FGGGTTLTVL
lv801       QLVLTQSPSASASLGASVKLTC  TLSSGHSSYAIA WHQQQPEKGPRYLMK LNSDGSHSKGD GIPDRFSGSSSGAERYLTISSLQSEDEADYYC QTWGTGIH
ASSynl190   ......................  .......T.... ......A........ ..T........ ............................... ...D...    QV FGGGTKLTVL

VλV         |<-      FR 1        ->|<-  CDR 1  ->|<-     FR 2  ->|<- CDR2  ->|<-           FR3                ->|< CDR 3>|
5c1         QAVLTQPASLSASPGASASLTC TLRSGINVGTYRIY WYQQKPGSPPQYLLR YKSDSDKQQGS GVPSRFSGSKDASANAGILLISGLQSEDEADYYC MIWHSSAS
ASSynL44    ......................  ........D..... .............S*

VλVI        |<-      FR 1        ->|<-  CDR 1  ->|<-     FR 2  ->|<CDR2->|<-             FR3              ->|<-    CDR 3  ->|<-  FR4 ->|
6s1         NFMLTQPHSVSESPGKTVTISC TRSSGGSIASNYVQ WYQQRPGSSPTTVIY EDNQRPS GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC QSYDSSNH
ASSynL195   ......................  .....N...D... ..........I... ....... ...................N.............. ......      IWV FGGGTKVTVL
BCSynL1     ...............E..V...  ............. ........A..A... ..DR... ...........T...A...I....RA........ ...S.     GSSVV FGGGTTLTVL
BCSynL3     ............F.........  .......V..... ........A...... ..T..Y. .I.........R.....A....R...D....... .....T..     VI FGGGTKLTVL
BCSynL38 ‖  ...........G...T......  .......D..... ..HX....A....V. ....... .......................E........... .....TY.     VI FGGGTKLTVL
BCSynL59    ...........G...T......  ..T.......F.. ...L....A...I.. ....... ..................................... ....TR.     PAV FGGGTRLTVL
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Rheumatoid Arthritis PBL

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VλIII       |<-      FR 1        ->|<- CDR 1 ->|<-     FR 2  ->|<CDR2->|<-           FR3              ->|<-    CDR 3    ->|<-  FR4 ->|
IGLV3S2     SYVLTQPPSVSVAPGKTARITC GGNNIGSKSVH WYQQKPGQAPVLVIY YDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
ASPBLL1     ...............Q......  ........... ...........V... ..R.... ................S.......V......H. ....WT...    KDIV FGGGTKLTVL
BCPBLL11    ..L...................  ........... ............S H.T.... ..................................  .....T...      YV FGTGTKVAVL
lv318       SYVLTQPPSVSVAPGKTARITC GGNNIGSKSVH WYQQKPGQAPVLVVY DDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
ASPBLL3     ....................P.  ..D....R..Q ..Y............ ...A... .............M.....TW.G......... E...TTTE.      VV FGGGTKLTVL
ASPBLL8     .....H................  ........... ............... ....... R............................... ..........      VV FGGGTKLTVL
ASPBLL23    ......................  ........... ............... ....... ..................................  ....NG....      WV FGGGTKLTVL
ASPBLL39    ....I.A.....S..E..T...  D.D...T.T.. .....L......... ....... ..SY.LA..K.A.......T..A......... ..........     LWV FGGGTKLTVL
2-14        SYVLTQPPSVSVAPGQTARITC GGNNIGSKSVH WYQQKPGQAPVLVVY DDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
BCPBLL3     ...................A.  .....A.... ............... ..N.... ..................................  ...E.....     NYV FGTGTKVTVL
BCPBLL5     ................T.S.  ..Y....R... ..........A..I. Y..... ..................................  ....T...      PDV FGTGTKVSVL
hsigll150   SYELTQPPSVSVSPGQTARITC SGDALPKQYAY WYQQKPGQAPVLVIY KDSERPS GIPERFSGSSSGTTVTLTISGVQAEDEADYYC QSADSSGTY
ASPBLL42    .F..N.......F.......P.  .A.V....FV.  .......H......F ..T.... W........N.....S.I.T........... .....N.      VLVV FGGGTKLTVL
ASPBLL48    ..D.............V....  .A.....RFT.  ..............F ..D.... .........Y.........T........E... .A..PT.R.      YV FGPGTTVSVL
ASPBLL51    ..D.............V....  .A.....RFT.  ..............F ..D.... .........Y.........T............ .A..PT.R.      YV FGPGTTVTVR
ASPBLL50    ......................  ........... ............... ....... ..................................  .........      PAV FGGGTKLTVL
ASPBLL53    .D....................  ...E...SFV. ....R........MK ..T.... ..................................  ...Y....      ALV FGGGTKLTVL
ASPBLL54 §  .....ATLG...PRTDGQDHLL CRCIAKAICLL VPAEARPGPCAGD.X RQX.ALR DPXAILWLQLRDNSHVDHQWSPGRRRGXLLLS I.RQQWYFPCGI    R RRDQADRPT
ASPBLL59    ......................  ......N..TS ...H......I...F R.R.... ...D.S.......IA.......R.......... ..PA.....      V FGSGTKVTLL
ASPBLL60    .H....................  .A.....KNT. ......D........ ....... ...A.....T..........A........... ..T.....F      V*
BCPBLL27    ......S...............  ........... .H...A........ ....... ..................................  .........      EV FGGGTKLTVL
3p          SYELTQPPSVSVSPGQTARITC SGDALPKKYAY WYQQKSGQAPVLVIY EDSKRPS GIPERFSGSSSGTMATLTISGAQVEDEADYYC YSTDSSGNHS
BCPBLL1     ..D...................  ........... ............... D.T.... ......................M........ .........      YV FGIGTKVTVL
BCPBLL25    ......................  ...D.SE.... ............V. ..K.... K............V..........D....... ......DA..     GI FGGGTKLTVL
BCPBLL26    ......................  ........... ............... ....... ..................................  .........      RV FGGGTKLTVL
BCPBLL28    ......................  ........... ............... ....... ...................*L......M....... .........      YV FGVGTEVTVL
BCPBLL31    ......A...............  ..N.......F ............... ...R... ...........................E... ....N...       PL FGGGTKLTVL
III.1       SYELTQPPSVSVSPGQTASITC SGDKLGDKYAC WYQQKPGQSPVLVIY QDSKRPS GIPERFSGSNSGNTATLTISGTQAMDEADYYC QAWDSSSTA
ASPBLL2     ................T.S.  .........T.  ............... ..IQ... ...................T....L....... ......      ALYV FGSGTRVTVL
ASPBLL4     ......................  .........TY ............... ....... ..................................  .......      LYV FGTGTKVTVL
ASPBLL10    PF.V..................  ....W.S..V. .F.........V.M. P.N.... .........K.............S......... .T..NDI.       V FGTGTKLTVL
ASPBLL12    ................T.S.  ........FV.  ............... .TF.... .T..........D................... ....N.I.        V FGGGTELTVL
ASPBLL13    ............L....T...  ..Y..E..FV.  .F......A...I.H ...Q... ...A..............N.I.TV....... .........      RV FGTGTKVFVL
ASPBLL14    ....S...A............  .......T.T. ............... ..TR... ..S......T.............S......... ....GR.       EV FGTGTKVTVL
BCPBLL2     ......................  ........... ............... E..... ..................................  .......      PWV FGTGTKVTVL
BCPBLL4     ...V..................  ......N...Y ............... E..... ..................................  ....NN.      PWV FGTGTKVTVL
BCPBLL6     ....F...............S.  ..E...E..V. ............... ..DR... ...P.............................  .........      V FGTGTKVTVL
BCPBLL7     ......................  ....V...... ............... ..N.... ..................................  .......     PSYV FGTGTKVTVL
BCPBLL8     .S.....H..........T.S.  ..FG...E..S .....S....L.... ..R.... .........K.E...N.......PT........ LM.N.     TSLFYV FG*
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Fig. 2 (Continued)

The presence of sequence variations that could represent allelic variants was not unexpected, as differences have been found in several germline VλIII gene segments from different individuals. These include the germline gene segments 3p/hsigll295 (4 nucleotide differences) and 3m/hsigll150 (3 nucleotide differences) (20). An informative example is the case of the closely related sequences humlv318 (29), 2–14 (21), and IGLV3S2 (35). The humlv318 and 2-14 gene segments differ by only one nucleotide in the coding sequence and are 4 nucleotides and 5

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BCPBLL9     ...................V.. ..........S ...L......L...S E.N.... .......A...............S........ ....G..        YV FGTGTKVTVL

VλI         |<-      FR 1        ->|<-   CDR 1  ->|<-     FR 2  ->|<CDR2->|<-             FR3            ->|<-  CDR 3  ->|<-  FR4 ->|
humlv1042   QSVLTQPPSVSGAPGQRVTISC TGSSSNIGAGYDVH WYQQLPGTAPKLLIY GNSNRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC QSYDSSLSGS
ASPBLL31    ..................... ...T.......... ............... ....... ...................I.......G.... ....      RLKV FGTGTRVTVL
ASPBLL34    .....H............... ...........A.. ............... A.T.... .....I...T.........S.......D.... ....Y.....  QV FGGGTKLTVL
ASPBLL35    ..................... ............... ............... ....... ................................ .........  LV FGGGTKLTVL

VλV         |<-      FR 1        ->|<-   CDR 1  ->|<-     FR 2  ->|<- CDR2  ->|<-             FR3             ->|<-  CDR 3  ->|<-  FR4 ->|
5a          QPVLTQPTSLSASPGASARFTC TLRSGINVGTYRIY WYQQKPGSLPRYLLR YKSDSDKQQGS GVPSRFSGSKDASTNAGLLLISGLQSEDEADYYC AIWYSSTS
BCPBLL13    .......S..........L.. ........APS... .............N ...G....E.. ...........V...................... ......     SWL FGGGTKLTVL
4-2         QAVLTQPSSLSASPGASASLTC TLRSGINVGTYRIY WYQQKPGSPPQYLLR YKSDSDKQQGS GVPSRFSGSKDASANAGILLISGLQSEDEADYYC MIWHSSAS
BCPBLL18    ............H.......S. ....D.D..F.... .F............. .R......EA. ..........I.........V............... .......      QV YGGGTKLTVL
5c2         QAVLTQPSSLSASPGASASLTC TLRSGINVGTYRIY WYQQKPGSPPQYLLR YKSDSDKQQGS GVPSRFSGSKDASANAGILLISGLQSEDEADYYC MIWHSSAS
BCPBLL14    ..................... ............... .....A........... ........... ................................ .......      WV FGGGTKLTVL
BCPBLL15    ...V................. ....D.S.TS.... ........S...... ..T....H... ................................F. .......      LV FGTGTKVTVL

VλVI        |<-      FR 1        ->|<-  CDR 1  ->|<-     FR 2  ->|<CDR2->|<-             FR3             ->|<-  CDR 3  ->|<-  FR4 ->|
6s1         NFMLTQPHSVSESPGKTVTISC TRSSGSIASNYVQ WYQQRPGSSPTTVIY EDNQRPS GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC QSYDSSNH
BCPBLL16    ..................... ............... ........A...... ....................................... ....G.   TFYV FGTGTKVTVL
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Normal PBL

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VλIII       |<-      FR 1        ->|<- CDR 1 ->|<-     FR 2  ->|<CDR2->|<-           FR3              ->|<-    CDR 3    ->|<-  FR4 ->|
IGLV3S2     SYVLTQPPSVSVAPGKTARITC GGNNIGSKSVH WYQQKPGQAPVLVIY YDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
ITPBLL56    ...............Q...... ..D...D.N.. ............... H...... ......................Q......... ....N.        VGV FGGGTKLTVL
2-14        SYVLTQPPSVSVAPGQTARITC GGNNIGSKSVH WYQQKPGQAPVLVVY DDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSDHP
LBPBLL2H    ......T....E.......... ...S....... ............... ..YR... .V.D........................... ....G....       FV FGSGTKVTVL
LBPBLL3     .........L.......... ......I.... ..........L..IH ..R.... .........D..D....I..G........... ......G..       VV FGGGTKLAVL
LBPBLL4D    ......T....E.......... ...S....... ............... ..YR... .V.D........................... ....G....       FV FGSGTKVTVL
ITPBLL1     ..................... ..D...RY... ............... ....... E........K...................... .....T...       YV FGTGTKVTVL
ITPBLL2     ....................S. ........... ............... ..N.... ...A..............S............ ...H.....       YV FGTGTKVTVL
ITPBLL28    ..................... E.K....TN.. .............F A...... ...................T...G........ ....GNENQ.       YV FGTGTKVTAL
ITPBLL66            *L...R...... ..HR...Y... ....RA......... ...G... ...........D..............G..... ....GH..Q.       YV FGTGTKVTVL
ITPBLL96    ....S................ ...Y....... .............F ....... A............................F. .....T...       QV FGGGTKLTVE

hsiql150    SYELTQPPSVSVSPGQTARITC SGDALPKQYAY WYQQKPGQAPVLVIY KDSERPS GIPERFSGSSSGTTVTLTISGVQAEDEADYYC QSADSSGTY
ITPBLL11    ..................... .........VH ............... ..N.... .....I..............R........... ..T......        R FGGGTKVTVL
ITPBLL12    ..................... ........... ............... ....... ................................ .........       WV FGGGTKLTVL
ITPBLL13    ..................... ........... ............... ....... ................................ ....Y...    SVNWV FGGGTKLTVL
ITPBLL15    ..................... ........NT. .............F E...... ................................ .........      IGV FGTGTKVTVL
ITPBLL16    ..................... ........... ............... ....... ................................ .........       QV FGGGTKLTVL
ITPBLL45    ................T.... ..AT......H ............M. R.K.... .............R.....T...D........ .T....     FERV FGTGTRVTVL
ITPBLL70    .H...........S........ ......N.FVF .....S....I.I.. R.T.... .....S...T...................... ....RGAH.        V FGTGTKLTVL
ITPBLL76    ......A............... ...GW.N.... ...........Q..F ..T.... ......Y..T...................... ..I.K...       VL FGRGTRLTVL
ITPBLL79    ..................... ........... ............... ....... ................................ .........       WV FGGGTKLTVL
ITPBLL80    ................S.... .........G. ............M. ..N..A. ................................ ....IT..      SVV FGGGTKLTVL
ITPBLL81    ..................... ........... ............... ....... ................................ .........      VV FGGGTKLTVL
LBPBLL2     ..................... .....T...SF .....A....A.L.. ..T.... .............KA....I............ ....N...      FWI FGGGTKLTVL
LBPBLL3F    ............R........ ...V.A...V. ............M. ..R.... .............A................... .T..R..     SFYV FGSGTKVTVV
LBPBLL3J    .F........T........... .....AD.... .....T.....V... ....... ................................ ......A.       VV FGGGTKLAVL
LBPBLL3K    .F........T........... .....AD.... .....T.....V... ....... ................................ ......A.       VV FGGGTKLAVL
LBPBLL3S    .F........T........... .....AD.... .....T.....V... ....... ................................ ......A.       VV FGGGTKLAVL
LBPBLL5     ......S............... ...V.A..H.. ...L........... R..Q... ........A...R....I............... ..S.NNVI          FGGGSKLTVL
LKPBLL9     ..................... ..E.LPNQYSY .....S........... ....... ................................ .........       KV FGGGTKLTVL
LKPBLL59    ..................... ........... ..........I.... ..T.... ................................ ....IR..       WV FGGGTKLAVL
LKPBLL70    ..................... ........... ............... ....... ................................ .........       WV FGGGTKLTVL
LKPBLL72    ..Q.................. ......S..T. .....S.....V.MF L.T.... .........T...................... ..P....I.        V SGTGTKVTVL
LKPBLL73    ..................... .........G. ............... ....... ................................ .........       WV FDGGTKLTVL
```

Fig. 2 (Continued)

nucleotides different from the IGLV3S2 gene segment, respectively. The finding that the humlv318 (3h) and 2–14 gene segments are located in the same position in the lambda locus on chromosome 2q11.2 in mapping studies (21,40) suggests that they represent allelic variants of the same gene segment. The fact that none of the five individuals in this study expressed both humlv318 and 2-14 (Table 1A) provides further support for this hypothesis.

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LKPBLL76    ..Q..................... ......R.... .....S.....V.M. L.T.... .........T....................... .......I.        V FGTGTKVTVL
3a          SYELTQPPSVSVSLGQMARITC SGEALPKKYAY WYQQKPGQFPVLVIY KDSERPS GIPERFSGSSSGTIVTLTISGVQAEDEADYYC LSADSSGTYP
ITPBLL86    ....................... ........... ............... ..R.... .............V.A................. .......S.      LYV FGTGTKVTVL
3p          SYELTQPPSVSVSPGQTARITC SGDALPKKYAY WYQQKSGQAPVLVIY EDSKRPS GIPERFSGSSSGTMATLTISGAQVEDEADYYC YSTDSSGNHS
LKPBLL69    ....................... ........... ............... ....... ................................ ........     LYVV FGGGTKLTVL
III.1       SYELTQPPSVSVSPGQTASITC SGDKLGDKYAC WYQQKPGQSPVLVIY QDSKRPS GIPERFSGSNSGNTATLTISGTQAMDEADYYC QAWDSSTA
ITPBLL22    ..................N.I. .A........S ...K......L...S ..T.... ............................F. ....R..        VV FGGGTKLTVL
ITPBLL24    .......A............S. ......*
ITPBLL25    ...............S.N... ..N...E.F.S ............... .GN.... ............D..S........L.....F. ........        GV FGGGTQLTVL
ITPBLL26                                                                                *T................... .....       KSYYV FGNGTKVTVL
ITPBLL92    ...................F.. ..H........ ........P...... ..T.... ................................ .......        VV FGGGTKLTVL
ITPBLL95    ....................... ........... ............... ....... .V............................... ......       INWV FGGGTKLTVL
ITPBLL97    ....................... ........... ............... .NN.... ................................ .....        GAYV FGTGTKVTVL
LBPBLL4F    ....................... ..........H ............... .GT.... ................................ ....       INWRV FGGGTKLTVL
LBPBLL4H    .........M........T... ..H...T.... ...........M... ..NN... ...D...............V............ .....        TFYV FGPGTKVTVL
LBPBLL4S    ....................... ........... ............... ....... ...................I........... .....N..        V FGGGTKLTVL
LKPBLL25    ..D.............VT... ..YN..N.... .....L......... ..T.... ........A..........T...T........ .T.....       PKYV FGTGTKVTVL
LKPBLL83    ....................... ........... ............... ....... ................................ .......        VV FGGGTKLTVL
LKPBLL84    ......S............... ...R.....V. ............... .NN.... ................................ .....N.        GV FGTGTKVTVL
LKPBLL85    ....................... ........... ..........VI.. ..N.... ................................ .....IS.       YV FGTGTKVTVL
LKPBLL87    ..D.....A...........S. ..H..E..... ......N.....IV. E.K...T ................N...A..L....... ....RT.        VV FGGGTKLTVI
LKPBLL88    ....................... ......N.... ............... ..N.... ................................ .......        GV FGTGTKVTVL
LKPBLL89                                                                                *Q.....R....... ....AG..        V FGTGTRVIVL
            |<-      FR 1        ->|<-  CDR 1 ->|<-     FR 2  ->|<CDR2->|<-           FR3            ->|<-    CDR 3   ->|<-  FR4 ->|
2-19        SYELTQPSSVSVSPGQTARITC SGDVLAKK-YAR WFQQKPGQAPVLVIY KDSERPS GIPERFSGSSSGTTVTLTISGAQVEDEADYYC YSAADNNL
LKPBLL68    .S.....A..........S... ........K... .L..R......V... ..I.... .........T...................... ...T...        WV FGGGTKLTVL

VλI         |<-      FR 1        ->|<-   CDR 1  ->|<-     FR 2  ->|<CDR2->|<-             FR3            ->|<-  CDR 3   ->|<-  FR4 ->|
humlv1042   QSVLTQPPSVSGAPGQRVTISC TGSSSNIGAGYDVH WYQQLPGTAPKLLIY GNSNRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC QSYDSSLSGS
LBPBLL2N    ....................... ............... ...........I... ....... ................................ ....NT....  YV FGTGTKVTVL
LKPBLL2     ....................... ............... ............... ....... ................................ .........  WV FGGGTKLTVL
LKPBLL58    .Y........Y........... ............... ....V.......... ....... ....G.L...W...................... ..........  LA FGGGTKLTVL

VλIV        |<-      FR 1        ->|<-  CDR 1 ->|<-     FR 2  ->|<- CDR2  ->|<-           FR3              ->|<- CDR 3  ->|<-  FR4 ->|
4a          QPVLTQSSSASASLGSSVKLTC TLSSGHSSYIIA WHQQQPGKAPRYLMK LEGSGSYNKGS GVPDRFSGSSSGADRYLTISNLQLEDEADYYC ETWDSNT
LKPBLL10  § ....................... ........... ..SSSQ.RPLGTXXS .KVVEATTR.A EFLIASQAPAL.LTGTSPSPTSS.RMRLIITV RPGTVTLG   V FGGGTKLTVL
5-4         QPVLTQSSSASASLGSSVKLTC TLSSGHSSYIIA WHQQQPGKAPRYLMK LEGSGSYNKGS GVPDRFSGSSSGADRYLTISNLQFEDEADYYC ETWDSNTH
LBPBLL2Q    ....................... .......NH... ............... ..S..R..Q.G .D.N...................SD.Q..... .....D.   RV FGGGTKVTVL
8A1         QLVLTQSPSASASLGASVKLTC TLSSGHSSYAIA WHQQQPEKGPRYLMK LNSDGSHSKGD GIPDRFSGSSSGAERYLTISSLQSEDEADYYC QTWGTGIH
LBPBLL2F    ....................... ........... ............... ....... ................................ ...A...   SV FGGGTKLTVL
lv801       QLVLTQSPSASASLGASVKLTC TLSSGHSSYAIA WHQQQPEKGPRYLMK LNSDGSHSKGD GIPDRFSGSSSGAERYLTISSLQSEDEADYYC QTWGTGIH
ITPBLL68    ....................... ........... ............... .......N... ................................ ...D..   FWV FGGGTKLTVL

VλV         |<-      FR 1        ->|<-   CDR 1  ->|<-     FR 2  ->|<- CDR2  ->|<-             FR3              ->|<-  CDR 3  ->|<-  FR4 ->|
5a          QPVLTQPTSLSASPGASARFTC TLRSGINVGTYRIY WYQQKPGSLPRYLLR YKSDSDKQQGS GVPSRFSGSKDASTNAGLLLISGLQSEDEADYYC AIWYSSTS
ITPBLL69    ...................I.. .V..V...DSC... ........P...... .......E... .......................WV........... .M...DV    YV FGTGTRVTVL
5c1         QAVLTQPASLSASPGASASLTC TLRSGINVGTYRIY WYQQKPGSPPQYLLR YKSDSDKQQGS GVPSRFSGSKDASANAGILLISGLQSEDEADYYC MIWHSSAS
LBPBLL7     ....................... ....D......... ..............S ......NH... ...........I.S.....V............... L....R.    YV FGTGTKVTVL
5c2         QAVLTQPSSLSASPGASASLTC TLRSGINVGTYRIY WYQQKPGSPPQYLLR YKSDSDKQQGS GVPSRFSGSKDASANAGILLISGLQSEDEADYYC MIWHSSAS
ITPBLL4   § ....................... ........... ............... ........... ................................ .......P  LGV RRRDQADRPT
LKPBLL52    ....................... ........D..... .F........L.... .N.....E... ................................ .....G.    YV FGTGTKVTVL
LKPBLL53  § ......S............... A..R.....S.... .....A.....H..T .......... .......................H......ND. ..RG        I RRRDQADRPT

VλVI        |<-      FR 1        ->|<-   CDR 1 ->|<-     FR 2  ->|<-CDR2>|<-             FR3              ->|<-    CDR 3  ->|<-  FR4 ->|
6s1         NFMLTQPHSVSESPGKTVTISC TRSSGSIASNYVQ WYQQRPGSSPTTVIY EDNQRPS GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC QSYDSSNH
LBPBLL4   § ....................... ............ ........A..I... .G.H... .........M..............R......... .....N.EA      V RRRDQADRPT
LBPBLL2S    T.I.................... .........DF.. ........A...... ..Y.... ......F.................. ..S.TT.       QV FGGGTRLTAL
ITPBLL75  § ....................... .G.G......FE. ......D.V.SI.V. D...... ..............Y.........E.---- VSLM MAGII    MS SKLRPRSPSY
```

Fig. 2 (Continued)

Variability of Jλ Gene Segment Expression in RA, and Expression of a Recently Described Jλ3 Allelic Variant

In humans, there are four functional λ isotypes encoded by the Cλ1, Cλ2, Cλ3, and Cλ7 genes, each with its own Jλ gene segment (21,48). Although the Jλ1, Jλ2, Jλ3, and Jλ7 gene segments (49–51) are the only expressed Jλ gene segments, Jλ6 is potentially functional (52). The Jλ2 and Jλ3 gene segments have previ-

Rheumatoid Arthritis Synovia

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Vλ III                    Vλ CDR3                    P      N      P                    Jλ                                  CDR3
Codon        89  90  91  92  93  94  95 95A 95B 95C |                |   96  97  98  99 100 101 102 103 104 105 106 107   Length
IGLV3S2      CAG GTG TGG GAC AGT AGT AGT GAT CAT CC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ASSynL220    ... ... ... ..T .C. G.. ... .C. ...    |                |   ... ... ... ... ... ... ... ... ... ... .C. ...      11
BCSynL4      ... ... ... ..T .A. .C. GC. ... ..     |                . ... ... ... ... GG. ... ... ... ... ... ... ...      11
BCSynL21     ... ... ... ..T .C. ..A .C. ... ..     |                . ... ... ... ..G ... ... ... .GC C.. ... ... ...      11
BCSynL22     ... ... ... ..T .C. ..A .C. ... ..     |                . ... ... ... ..G ... ... ... .GC C.. ... ... ...      11
BCSynL24     ... ... ... ..T ... ... ... ... ..     |                . ... ... ... ... ... ... ... ... ... ... ... ...      11
                                                    |                T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASSynL182    ... ..A ... ..T .T. .C. ... ... .C.    |                  ... A.. ... ... ... ... ... ... ... ... ... ...      11
ASSynL189    ... ... ... ..G ... ... ... ... ...    |                  ... ... ... ... ... ... ... ... ... ... ... ...      11
                                                    |                T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL232    ... ... ... .TT C.. GA. TC. ... ..     |       GC         .. C.. ... ... ... ... ... ... ... ... ... ..C      11
ASSynL234    ... ... ... ..T .A. .A. ... ... ...    |                  ... ... ... ... ... ... ... ... ... ... ... ...      11

humlv318     CAG GTG TGG GAT AGT AGT AGT GAT CAT CC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ASSynL186    ... ... ... ... ... ... ... ..C .     |       C          . ... ... ... ... .G. ... ... .T. ... ... ... ...      11
ASSynL221    ... ... ... ... .A. .AG G.             |       G              ... ... ... ... ... ... ... ... ... ... G..       8
ASSynL236    ... ... ... ... .C. ... ... .C. ..     |                . ... ... ... ... ... ... ... ... ... ... .C. ...      11
                                                    |                T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
BCSynL36     ... ... ... ..A .T. ... ... ... ...    |                  ... ... ... ... ... ... ... ... ... ... ... ...      11

hsigll150    CAA TCA GCA GAC AGC AGT GGT ACT TAT CC |                | T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASSynL202    ... ..G ..T ... .A. ..C ..C C.. ...    |       TA         . ... .CT ... ... ... ... ... .G. ... ... ... ..G      12
ASSynL210    ... ... ..G ... .T. .C. AA. ... .      |        G           . ... ... ... ... ... ... ..A ... ... ... ..C      10
ASSynL215    ... ... A.. .G. ... ... ... C.. ...    |                  ... ..G ... ... ... ... ... .G. ... ... ... ...      11
ASSynL216    ... ... .T. ... ..T ..A ..A .          |       AA         ... .AT ... ... ... ... ... ..A T.. ... ... ...      10
ASSynL217    ... ... ... ... ... ... ... ..         |      CCCCC           .. ... ... ... ... ... ... ... ... ... ...      10
BCSynL17     ... ... ... ... .T. ... ..A ... .C.    |                  ... A.. ... ... ... ... ... ... ... ... ... ...      11
BCSynL18     ... ... ... ... .A. .A. ... GG. ... .  |       AAAG         . A.T ... ... ... ... ... ... ... ... ... ..G      12
BCSynL47     ... ... ... ... .CG ..G ..C ..         g     ATA              ... ..T ... ... ... ... ... T.. ... ... ..G      10
BCSynL51     ..G ... A.. ... .CT .C. ..G .A. .TA .  |                       .T ... ... ... ... ... ..A ... ... ... ..C      10
                                                    |                T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL204    ... ... ... ... ..G ... ... ...        |                  ... ... ... ... ... ... ... ... ... ... ... ...      10
ASSynL214    G.. ... .AG ... ... ... ... TT.        |                  ... ... ... ... ... ... ... ... G.. ... ... ...      10
ASSynL209*   ... ... ... ... ... GA. ... T.. ... .  |        A           . ... ... ... ... ... ... ... T.. ... ... ..G      11
ASSynL211*   ... ... ... ... ..A GA. ..G T.. ... .  |        A           . ... ... ... ... ... ... ... T.. ... ..T ...      11
ASSynL184*   ..G ... ... ... ..A CT. ... ... ... .  |        A           . ..C ... ... ... ... ... C.C ... ..T ... T..      11

3p           TAC TCA ACA GAC AGC AGT GGT AAT CAT AG |                | T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
BCSynL16*    ... ... ... ... ... ... ... G.G .      |       T       c  ... ... ... ... ... ... ... ... .GA ... ... ... ..C      11
BCSynL52*    ... ... ... ... ... ... ... G.G .      |       T       c  ... ... ... ... ... ... ... ... .GA ... ... ... ..C      11
BCSynL53*    ... ... ... ... ... ... ... G.G .      |       T       c  ... ... ... ... ... ... ... ... .GA ... ... ... ..C      11
                                                    |                T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL136    ... ... ... ... ... G.. .A. ... ... .. |       A              ... ... ... ... ... ... ... ... ... ... ... ...      11
BCSynL46     ... ... ... ... ... ... ... G.. ..A .  |                  .. ... ... ... ... ... ... ... ... ... ... ... ...      11

2-19         TAC TCT GCG GCT GAC AAC AAT CT         |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL201    ... ... ... ... ... ... ... ..         |        GG        .. ... ... ... ... ... ... ... ... ... ... ... ...      10

III.1        CAG GCG TGG GAC AGC AGC ACT GCA        |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ASSynL226    ... ... ... ... ... ..G .              |       AGAT         . ... ... ... ... ... ... ... ... ... ... ... ...       9
ASSynL235    ... ... ... ... ... ... ... ..         g                     ... ... ... ... ... ... ... ... ... ... ... ...       9
BCSynL65     ... ... ... ... ... G.. ... .G.        |                     ... ... ... ... ... ... ... ... ... ... ..T T..       9
                                                    |                T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASSynL25     ... A.. ... ... .A. ..         |TTCACCGTGGGACAACGAGACTGCA| .C. ..T ... ... ... ... ... ... ... ... ... .GT      16
ASSynL227    ... ... ... ... .C. .AT .              t     G              .G. ..C ... ... ... ... ... ... T.. ... ... ...       9
ASSynL230    ... ... ... ... ... ... ... .          |                  .. ..T ... ... ... ... ... ..A ... ... ... ...       9
BCSynL56     ... .T. ... ... ... ... .              t                . ... ..T ... ... ... ... ... ... ... ... ... ...       9

Vλ I                      Vλ CDR3                                                   Jλ
Codon        89  90  91  92  93  94  95 95A 95B 95C |                |   96  97  98  99 100 101 102 103 104 105 106 107
humlv1042    CAG TCC TAT GAC AGC AGC CTG AGT GGT TC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
BCSynL32     ... ..G ..C ... ... ... ... ... ... .  |       GG     aa  . ... ... ... ..C ... ... ... ... ... ... ... ...      13
                                                    |                T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASSynL188    ... ... ... ... .T. ... ... ... ... .. |g                 .G. ..T ... ... ... ... ... ... ... ... ... ..T      12

DPL6         CAG TCC TAT GAC AGC AGC CTG AGT GGT TC |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL191    ... ... ... ... ... ... ... ... .A. .. |              c    ... ... ... ... ... ... ... ..A G.. ... ... ...      11
```

Fig. 3. Nucleotide sequences of Vλ-Jλ joins of cDNA clones amplified from RA synovia, RA PBL, and normal PBL. Dots indicate sequence homology. Palindromic (P) nucleotides are shown in lower-case letters. *, related sequences (see text for details). Clone BCPBLL8 is truncated in the Jλ gene segment. §, Clones with insertions in CDR3 intervals: LKPBLL10 contains a one-nucleotide deletion in FR2 and a one-nucleotide insertion in CDR3; LKPBLL53, ITPBLL4, and LBPBLL4 contain single nucleotide insertions in CDR3 intervals. The sequence at the Vλ-Jλ join in ASSynL25 may represent an insertion event from codons 89 through 95A of the germline VλIII.1 CDR3 component. Nucleotides shared by the junctional sequence of ASSynL25 and germline VλIII.1 are underlined (see Discussion for details). The number of codons in CDR3 intervals are shown.

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VλIV                    Vλ CDR3                                                                  Jλ
Codon        89  90  91  92  93  94  95 95A |                  |   96  97   98  99 100 101 102 103 104 105 106 107
8A1          CAG ACC TGG GGC ACT GGC ATT CA |                  | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
BCSynL37     ... ... ... ... ...            |        TA        | . ..T A..  ... ... ... ... ... .C. ... ... ... ..G      8
                                            |                  | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL59     ... ..G ... ... ... ... .G. .. |                  | . ... ...  ... ... ..C ... ... .C. ... ... ... T..     10

humlv801     CAG ACC TGG GGC ACT GGC ATT CA |                  | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL190    ... ... ... .A. ... ... ... .. |                  |   . ...    ... ... ... ... ... ... ... ... ... ...      9

VλVI                    Vλ CDR3                                                                  Jλ
Codon        89  90  91  92  93  94  95 95A |                  |   96  97   98  99 100 101 102 103 104 105 106 107
lv6S1        CAG TCT TAT GAT AGC AGC AAT CA |                  | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
BCSynL1      ... ... ... AG. ..T G.. .G.    |        TC      c |   ... ..G  ..T ... ... ... ..G .CA ... ... ... ...     10
BCSynL3      ... ... ... ... ... .C. ... .. |                  | . ... A..  ... ... ... ... ... ... ... ... ... ...     10
BCSynL38     ... ... ... ... ... .C. T.. .. |                  | . ... A..  ... ... ... ... ... ... ... ... ... ...     10
BCSynL59     ... ... ... ... .C. ..G ..C .  |        C         | . .CA ..T  ... ... ... ... ... .G. T.. ... ... ...     10
                                            |                  | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASSynL195    ... ... ... ... ... ... .T.    |                  |   ... ...  ... ... ... ... ... ... G.. ... ... ...      9
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VλIII                   Vλ CDR3                      P      N      P                  Jλ
Codon        89  90  91  92  93  94  95 95A |                  |   96  97   98  99 100 101 102 103 104 105 106 107
IGLV3S2      CAG GTG TGG GAC AGT AGT AGT GAT CAT CC |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
BCPBLL11     ... ... ... ..T ... .C. ... ... ...    |          |   ... ...  ... ... ... ... ... ... ... G.. ... ...     11
                                                    |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASPBLL1      ..A ... ... ..T T.G .C. ... ... ...    | a    AGG   a | . A.C ...  ... ... ... ... ... ... ... ... ... ...     13

humlv318     CAG GTG TGG GAT AGT AGT AGT GAT CAT CC |          |   T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
BCPBLL3      ... ... ... ..A ... ... ... ... ...    | a        a |   . ... ...  ... ... ... ... ... ... ... ... ... ...     12
BCPBLL5      ... ... ... ... .C. ... ... ... .      |      C   |   . G.C ...  ... ... ... ... ... ... ... T.. ... ...     11
                                                    |          |   T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASPBLL3      G.. ... ... ... .C. .CC .C. ..A ..     |          |   . ... ..C  ... ... ... ... ... ... ... ... ... ...     11
ASPBLL8      ... ... ... ... ... ... ... ... ... .. | g        |     ... ...  ... ... ... ... ... ... ... ... ... ...     12
                                                    |          |   T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASPBLL23     ... ... ... ... .A. G.C ... ... ... .. |          |   . ... ...  ... ... ... ... ... ... ... ... ... ...     12
ASPBLL39     ... ... ... ... ... ... ... ... ... .  |      TG  |     ... ...  ... ... ... ... ... ... ... ... ... ...     12

hsigll150    CAA TCA GCA GAC AGC AGT GGT ACT TAT CC |          |   T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ASPBLL48*    ..G G.T ... ... CC. .C. ..A CGC ...    |          |     ... ...  ... ... C.. ... ..G .CA ... T.. ... ..G     11
ASPBLL51*    ..G G.T ... ... CC. .C. ... CGC ...    |          |     ... ...  ... ... C.. ... ..G .CA ... ... ... .GG     11
ASPBLL59     ... ..G C.. .C. ... ... ... ..         |          | . ... ...  ... ... .G. ... ... ... ... ... C.. ...     10
                                                    |          |   T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASPBLL42     ... ... ..G ... ..T .A. ..A GT.        |      CT  | . ... ..C  ... ... ... ... ... ... ... ... ... T..     11
ASPBLL50     ... ... ... ... ... ... ... ... ... .. | g        |   .C. ..G  ... ... ... ... ... ... ... ... ... ...     12
ASPBLL54     ... ... ... ... ... ... ... ... .CC .  |          | a | . ... ...  ... ... ... ... ... ... ... ... ... ...     12
ASPBLL60     ... ... A.. ... ... ... ... ... .T. G*
BCPBLL27     ... ... ... ... ... ... ... ... ...    |          |   .AA ..G  ... ... ... ... ... ... ... ... ... ...     11
                                                    |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASPBLL53     ... ... ..G T.. ... ... ..A G.         |      C   |   ... ...  ... ... ... ... ... ..A ... ... ... ...     10

3p           TAC TCA ACA GAC AGC AGT GGT AAT CAT AG |          |   T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
BCPBLL1      ... ... ... ... ... ... ... ... ...    |          |   ... ...  ... ... .T. ... ... ... ... ... ... ...     11
BCPBLL28     ... ... ... ... ... ... ... ... ...    |          |   ... ..T  ... ... GT. ... ... G.. ... ... ... ...     11
                                                    |          |   T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
BCPBLL25     ... ... ... ... ... ... .A. GCC ... .. | c    GG  aa |       .T  ... ... ... ... ... ... ... ... ... ...     12
BCPBLL26     ... ... ... ... ... ... ... ... ... .. |          |     . ...  ... ... ... ... ... ... ... ... ... ...     11
BCPBLL31     ... ... ... ... .A. ... ... ... .      |     CCC  |       ..   ... ... ... ... ... ... ... ... ... ...     10

III.1        CAG GCG TGG GAC AGC AGC ACT GCA        |          |   T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ASPBLL2      ... ... ... ... ... ... G..            |      CT  | . ... ...  ... ..G T.. ... ... .G. ... ... ... ..C     10
ASPBLL4      ... ... ... ... ... ... ...            |      CT  | . ... ...  ... ... ... ... ... ... ... ... ... ...     10
ASPBLL10     ..A A.. ... ... .A. GA. .T. ..         |          |     . ...  ... ... ... ... ... ... C.. ... ... T..      9
ASPBLL13     ... ... ... ... ... ... ... ..         | g   AGG  |       ...  ... ... ... ... ... ... ... TT. ... T.G     10
ASPBLL14     ... ... ... ... G.. ..A ..C .          |          |   .G ...   ... ... ... ... ... ... ... ... ... ..T      9
BCPBLL2*     ... ... ... ... ... ... ... C.         |          | . .GG ...  ... ... ... ... ... ... ... ... ... ...     10
BCPBLL4*     ... ... ... ... .A. .A. ... C.         |          | . .GG ...  ... ... ... ... ... ... ... ... ... ...     10
BCPBLL6      ... ... ... ... ..T ... ... ..         |          |     . ...  ... ... ... ... ... ... ... ... ... ...      9
BCPBLL7      ... ... ... ... ... ... ... C.         |     TTC  | . ... ...  ... ... ... ... ... ... ... ... ... ...     11
BCPBLL8      .T. AT. ... A.. ..T .C. .G.            |   TTGTT  | . ... ...  ... ...*                                   11
BCPBLL9      ... ... ... ... G.. ... ...            |          |   ..C ...  ... ... ... ... ... ... ... ... ... ...      9
                                                    |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASPBLL12     ... ... ... ... .AT ... .T. ..         |          |     . ..C  ... ... ... ... ... G.. ... ... ... ...      9
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Fig. 3. (Continued)

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VλI                    Vλ CDR3                                                                                  Jλ
Codon        89  90  91  92  93  94  95 95A 95B 95C |                |   96  97  98  99 100 101 102 103 104 105 106 107
humlv1042    CAG TCC TAT GAC AGC AGC CTG AGT GGT TC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ASPBLL31     ... ... ... ... C.                     |     GCTGA      |   .G ...  ... ..G ... ... ... .G. ... ... ... ..G      8
                                                    |                | T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ASPBLL35     ... ... ... ... ... ... ... ... ... .  |                |   .. ...  ... ... ... ... ... ... ... ... ... ...     11
                                                    |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ASPBLL34     ... ... ... ... TA. ... ... ... ..G .. |g    CA         |    . ...  ... ... ... ... ... ... ... ... ... ...     12

VλV                    Vλ CDR3                                                                                  Jλ
Codon        89  90  91  92  93  94  95 95A         |                |   96  97  98  99 100 101 102 103 104 105 106 107
5a           GCC ATT TGG TAC AGC AGC ACT TCT        |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
BCPBLL13     ... ... ... ... ... ... T..            |                |   ... C.. ... ... ... ... ... ... ... ... ... ..T      9

4-2          ATG ATT TGG CAC AGC AGC GCT TCT        |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
BCPBLL18     ... ... ... ... ..T ... ...            |     CA         |    . ...  .A. ... ... ... ... ... ... ... ... ...      9

5c2          ATG ATT TGG CAC AGC AGC GCT TCT        |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
BCPBLL15     ... ..C ... ... ... ... ..             |     CCTG       |       ...  ... ... ... ... ... ... ... ... ... ...      9
                                                    |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
BCPBLL14     ... ... ... ... ... ... ...            |                |   ... ...  ... ... ... ... ... ... ... ... ... ...      9

VλVI                   Vλ CDR3                                                                                  Jλ
Codon        89  90  91  92  93  94  95 95A         |                |   96  97  98  99 100 101 102 103 104 105 106 107
lv6S1        CAG TCT TAT GAT AGC AGC AAT CA         |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
BCPBLL16     ... ... ... ... G.. ... .              |     CCTT       | . ... ...  ... ... ... ... ... ... ... ... ... ...     10
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Normal PBL

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VλIII                  Vλ CDR3                      P     N      P                                                   Jλ
Codon        89  90  91  92  93  94  95 95A 95B 95C |                |   96  97  98  99 100 101 102 103 104 105 106 107
IGLV3S2      CAG GTG TGG GAC AGT AGT AGT GAT CAT CC |                | T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ITPBLL56     ... ... ... ... .A. ...                |     GTAGG      |    . ..C  ... ... ... ... ... ... ... ... ... ...      9

humlv318     CAG GTG TGG GAT AGT AGT AGT GAT CAT CC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ITPBLL1      ... ..A ... ... ... .C. ... ... ..     |                | . ... ...  ... ... ... ... ... ... ... ... ... ...     11
ITPBLL2      ... ... ... C.. ... ... ... ... ..     |                | . ... ...  ... ... ... ... ... ... ... ... ... ...     11
ITPBLL28     ... ... ... ... G.. .A. GAA A.. ..G .. |                | . ... ...  ... ... ... ... ... ... ... ... .C. ..C     12
ITPBLL66     ... ... ... ... G.. CA. ... ... ..G .. |                | . ... ...  ... ... ... ... ..T ... ... ... ... ...     12
LBPBLL2H*    ... ... ... ... G.A ..C ... ... ..     |                | . .T. ..T  ... ... .G. ... ... ... ... ... ... ...     11
LBPBLL4D*    ... ... ... ... G.A ..C ... ... ..     |                | . .T. ..T  ... ... .G. ... ... ... ... ... ... ...     11
                                                    |                | T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LBPBLL3      ... ... ... ... ... ... G.. ... ..     |                | . ... ..G  ... ... ... ... ... ... ... G.. ... ...     11
ITPBLL96     ... ... ... ... ... .CG ... ... ... .  |     A          |    . ...  ... ... ... ... ... ... ... ... ... GA.     11

hsigll150    CAA TCA GCA GAC AGC AGT GGT ACT TAT CC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
LBPBLL3F     ..G A.. ... ... ..A ... ... .G. .      |     T          | . ... ...  ... ... .G. ... ... ... ... ... ... G..     11
ITPBLL15     ... ... ... ... ... ... ... ...        |a    TAGGG      |       ...  ... ... ... ... ... ... ... ... ... ...     11
ITPBLL45     ... A.. ..C ... ... TT. .AG .          |     GG         |       ...  ... ... ... ... ... .G. ... ... ... ...      9
ITPBLL70     ... ..G ... ... ..A G.. .C.            |     C       a  | . ... ...  ..T ... ... ... ... ... C.. ... ... ...     10
LKPBLL72*    ... ... C.. ... ... ... ... .          |t                | . ... ...  .C. ... ... ... ... ... ... ... ... ...     10
LKPBLL76*    ... ... ... ... ... ... ... .          |t                | . ... ...  ... ... ... ... ... ... ... ... ... ...     10
                                                    |                | T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LBPBLL2      ..G ... ... ... .A. ... ... ... .      |     TCTG       |    . A.C  ... ... ... ... ... ... ... ... ... ...     11
LBPBLL3J*    ... ... ... ... ..T ..C .C. ..         |                | . ... ..T  ... ... ... ... ... ... ... G.. ... ...     10
LBPBLL3K*    ... ... ... ... ..T ..C .C. ..         |                | . ... ..T  ... ... ... ... ... ... ... G.. ... ...     10
LBPBLL3S*    ... ... ... ... ..T ..C .C. ..         |                | . ... ..T  ... ... ... ... ... ... ... G.. ... ...     10
LBPBLL5      ... ... T.C ... .A. .                  |       a        | . ..T A..  ... ... ... ... T.. ... ... ... ... ...      8
ITPBLL11     ... ... A.. ... ... ... ... ... ... .  |g               |         .  ... ... ... ... ... ... G.. ... ... ...     10
ITPBLL76     ... ... ATT ... .AA ... ..C ..         |       c        |   ... T..  ... ... A.. ... ... .G. ... ... ... ...     10
ITPBLL80     ... ... ..G ... .T. .C. ... ..C .      |     C       c  |   ... ...  ... ... ... ... ... ... ... ... ... ...     11
ITPBLL81     ... ... ... ... ... ... ... ... ..     |                | . ... ...  ... ... ... ... ... ... ... ... ... ...     11
                                                    |                | T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ITPBLL12     ... ... ... ... ... ... ... ...        |                |   ... ...  ... ... ... ... ... ... ... ... ... ...     10
ITPBLL13     ... ... ... ... TA. ... ... ... .      |     CCGT   caa | . ... ...  ... ... ... ... ... ... ... ... ... ...     13
ITPBLL16     ... ... ... ... ... ... ... ... ... .  |     A          |    . ...  ... ... ... ... ... ... ... ... ... ...     11
ITPBLL79     ... ... ... ... ... ... ... ... ...    |                |   ... ...  ... ... ... ... ... ... ... ... ... ...     11
LKPBLL9      ... ... ... ... ... ... ... ... ...    |a    A          |    . ...  ... ... ... ... ... ... ... ... ... ...     11
LKPBLL70*    ... ... ... ... ... ... ... ...        |                |   ... ...  ... ... ... ... ... ... ... ... ... ...     10
LKPBLL73*    ... ... ... ... ... ... ... ...        |                |   ... ...  ... .A. ... ... ... ... ... ... ... ...     10
LKPBLL59     ..G ... ... ... .T. ..G ... ...        |                |   ... ...  ... ... ... ... ... ... T.. G.. ... ..T     10

3a           CTA TCA GCA GAC AGC AGT GGT ACT TAT CC |                | T TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ITPBLL86     ... ... ... ... ... ... ... T.. ... .  |     T          | . ... ...  ... ... ... ... ... ... ... ... ... ...     12
```

Fig. 3. (Continued)

```
3p          TAC TCA ACA GAC AGC AGT GGT AAT CAT AG |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LKPBLL69    ... ... ... ... ... ... ... ... .      |    TCTA  | . ... ...  ... ... ... ... ... ... ... ... ... ...      12

2-19        TAC TCT GCG GCT GAC AAC AAT CT         |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LKPBLL68    ... ... ... A.. ... ... ...            |      TG  |   . ...    ... ... ... ... ... ... T.. ... ... ..G       9

III.1       CAG GCG TGG GAC AGC AGC ACT GCA        |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
LBPBLL4H    ... ... ... ... ... .C.                |      TT  | . ... ...  ... ... C.. ... ... ... ... ... ... ...       9
ITPBLL26    ... ... ... ... ... .AG T..            |      TA  | . ... ...  ... ... .A. ... ... ... ... ... ... ...      10
ITPBLL97    ... ... ... ... ... G.. G.             |          | . ... ...  ... ... ... ... ... ... ... ... ... ...       9
LKPBLL25    ..A A.. ... ... ... ... ..C C.         |    TAAG  |   ... ...  ... ... ... ... ... ... ... ... ... ...      11
LKPBLL84    ... ... ... ... ... .A. ... .G.        |          |       ...  ... ... ... ... ... ... ... ... ... ...       9
LKPBLL85    ... ... ... ... ... .T. T.. ..         |          | . ... ...  ... ... ... ... ... ... ... ... ... ...      10
LKPBLL88    ... ... ... ... ... ... ... .G.        |          |       ...  ... ... ... ... ... ... ... ... ... ...       9
                                                   |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
ITPBLL22    ... ... ... ... ..A ..T ...            |          |   ... ...  ... ... ... ... ... ... ... ... ... ...       9
ITPBLL92    ... ... ... ... ... ... ..             |        g |   ... ...  ... ... ... ... ... ... ... ... ... ...       9
LKPBLL83    ... ... ... ... ... ... ..             |          | . ... ...  ... ... ... ... ... ... ... ... ... ...       9
LKPBLL87    ... ... ... ... ..G .CT ... .          |          |   .. ..C   ... ... ... ... ... ... ... ... ... A..       9
                                                   |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
LBPBLL4F    ... ... ... ... .T. .A.                |     TGGA |   .. ...   ... ... ... ... ... ... ... ... ... ...       9
LBPBLL4S    ... ... ... ... ... .A. ... ..         |          |    . ...   ... ... ... ... ... ... ... ... ... ...       9
ITPBLL25    ... ... ... ... ..T ... ... ..         |        T |   ... ...  ... ... ... ... ... C.A ... ... ... ..G      10
ITPBLL95    ... ... ... ... ... ... .              |        t |     caa| . ... ...  ... ... ... ... ... ... ... ... ... ...      10

VλI                     Vλ CDR3                                                   Jλ
Codon        89  90  91  92  93  94  95 95A 95B 95C|          |   96  97  98  99 100 101 102 103 104 105 106 107
humlv1042   CAG TCC TAT GAC AGC AGC CTG AGT GGT TC |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
LBPBLL2N    ... ..T ... ... .A. .C. ... ... ... .. |        g |   ... ...  ... ... ... ... ... ... ... ... ... ...      12

                                                   |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LKPBLL58    ... ... ... ... ... ... ... ... ... .. |      CC  |   .. .C.   ... ... ... ... ... ... ... ... ... ...      12
                                                   |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
LKPBLL2     ... ... ... ... ... ... ... ... ...    |          |   ... ...  ... ... ... ... ... ... ... ... ... ...      11

VλIV                    Vλ CDR3                                                   Jλ
Codon        89  90  91  92  93  94  95 95A         |          |   96  97  98  99 100 101 102 103 104 105 106 107
5-4         GAG ACC TGG GAC AGT AAC ACT CA         |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
LBPBL2Q     ... ... ... ... ... G.. ... .          |          |   .C ...   ... ... ... ... ... ..A G.. ... ... ...       9
LKPBLL10 §  ... ... ... ... ... ... ... .          |          |   ... ...  ... ... ... ... ... ... ... ... ... ...     9.3

8A1         CAG ACC TGG GGC ACT GGC ATT CA         |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LBPBL2F     ... ... ... .C. ... ... ..             |        a |     AGT  |       ...  ... ... ... ... ... ... ... ... ... ...       9

lv801       CAG ACC TGG GGC ACT GGC ATT CA         |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ITPBLL68    ... ... ... .A. ... ... T..            |          |   ... ...  ... ... ... ... ... ... ... ... ... ...       9

VλV                     Vλ CDR3                                                   Jλ
Codon        89  90  91  92  93  94  95 95A         |          |   96  97  98  99 100 101 102 103 104 105 106 107
5a          GCC ATT TGG TAC AGC AGC ACT TCT        |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ITPBLL69    ... ..G ... ... ... GA.                |       gt | . ... ...  ... ... ... ... ... .G. ... ... ... ...       9

5c1         ATG ATT TGG CAC AGC AGC GCT TCT        |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
LBPBLL7     C.. ... ... ... ... ..A ... .          |          |   .. ...   ... ... ... ... ... ... ... ... ... ...       9

5c2         ATG ATT TGG CAC AGC AGC GCT TCT        |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
LKPBLL52    ... ... ... ... ... G.. ..             |          | . ... ...  ... ... ... ... ... ... ... ... ... ...       9
                                                   |          | T GTG GTA  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ2
LKPBLL53 §  ... ... A..                            |          |   . ...    ... ... ... ... ... ... ... ... ... ...     4.3
                                                   |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
ITPBLL4  §  ... ... ... ... ... ... ..C C.         |      TT  |   ... ...  ... ... ... ... ... ... ... ... ... ...    10.3

VλVI                    Vλ CDR3                                                   Jλ
Codon        89  90  91  92  93  94  95 95A         |          |   96  97  98  99 100 101 102 103 104 105 106 107
lv6S1       CAG TCT TAT GAT AGC AGC AAT CA         |          | T TAT GTC  TTC GGA ACT GGG ACC AAG GTC ACC GTC CTA Jλ1
ITPBLL75    ... ... ... ... G.. ..G ... ..         |          | . ... ...  ... .A. ... .C. ... ... ... ... ... ...      10
                                                   |          | T TGG GTG  TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Jλ3
LBPBL2S     ... ... .C. ... .C. .C. ... ..         |          |   . ...    ... ... ... ... ... .GA ..C ... .C. ...       9
LBPBLL4  §  ... ... ... ... ... .A. ... G.         |      A   |   . C..    ... ... ... ... ... ... ... ... ... ...     9.3
```

Fig. 3. (Continued)

ously been reported to be identical in nucleotide sequence (30,50,53). In this study, a total of 158 clones contained identifiable Jλ gene segments (Table 1C, Fig. 4). Surprisingly, a variant Jλ3 gene segment (21), (Genbank Accession Number D87023) was found in 41 cDNA clones (26%) and was expressed in all five individuals in this study. The definitive

Table 1B. Germline derivation of unrearranged Vλ gene segments amplified from genomic DNA of rheumatoid arthritis patient BC and normal control LB

	Vλ Family	VλIIIa	VλIIIb			VλIIIc		
PBL	Gene Segment	3i	150	3a	3p	III.1	3e	Total
RA	BC	3	3	0	3	6	1	16
Normal	LB	1	2	1	3	8	2	17
	Total	4	5	1	6	14	3	33

All but two genomic sequences in Table 1B were 100% identical to reported sequences of corresponding germline gene segments (Genbank Accession numbers: LBLG9-AF063764; LBLG14; AF063763). Mismatches in number of clones analyzed in Tables 1A and 1B are attributable to truncated sequences.

evidence of expression of what is presumed to be an allelic variation of the Jλ3 gene segment supports our contention that shared changes in Vλ gene segments represent novel allelic variants.

Because the downstream PCR primer used for cDNA amplification was derived from a conserved region of the constant region, Jλ gene segment utilization in different samples could be compared without concern for bias because of preferential Jλ amplification. The pattern of Jλ expression was similar in the

Table 1C. Germline derivation of Jλ gene segments in cDNA clones

	Rheumatoid Arthritis				Normal			
Gene Segment	AS Synovium	AS PBL	BC Synovium	BC PBL	LB PBL	IT PBL	LK PBL	Total
Jλ1	6 (20.0%)	9 (40.9%)	6 (28.6%)	13 (65.0%)	6 (33.3%)	12 (42.8%)	7 (36.8%)	59 (37.3%)
Jλ2	11 (36.7%)	9 (40.9%)	13 (61.9%)	4 (20.0%)	7 (38.9%)	8 (28.6%)	6 (31.6%)	58 (36.7%)
Jλ3	13 (43.3%)	4 (18.2%)	2 (9.5%)	3 (15.0%)	5 (27.7%)	8 (28.6%)	6 (31.6%)	41 (26.0%)
Total	30	22	21	20	18	28	19	158

Table 2. Possible variants of known germline Vλ gene segments expressed in cDNA clones from patients with rheumatoid arthritis and normal controls

Gene Segment	Codon	Domain	Reported Sequence	Variant Sequence	Non-clonally Related Sequences Containing Described Variation	% of clones with variant sequence
IGLV3S2	92	CDR3	GAC	GAT	ASSynL182, 232; ASPBLL1, 220, 234; BCPBLL11; BCSynL4, 21, 22, 24	83.3%
hsigll150	32	CDR1	TAT	TTT	ASSynL202, 209; ASPBLL42, 53; BCSynL47; ITPBLL70	14.3%
hsigll150	33	CDR1	GCT	GTT	ASSynL216; ASPBLL42, 53; BCSynL51; LBPBLL3F; LKPBLL9; ITPBLL11, 70	17.8%
hsigll150	46	FR2	CTG	TTG	ASSynL204, 215, 216; ASPBLL48, 51, 59; BCSynL47; BCPBLL27; LBPBLL2, 3F, 5; LKPBLL9, 59; ITPBLL13, 45, 70, 80	40.0%
hsigll150	52	CDR2	AGT	ACT	ASSynL209, 210, 211, 215, 216; ASPBLL42 53; LBPBLL2; LKPBLL59, 76, 72; ITPBLL70, 76	28.9%
hsigll150	87	FR3	TAC	TAT	ASSynL84, 202, 209, 211, 214, 216; ASPBLL42, 53; BCSynL17, 51; BCPBLL27; LBPBLL3J, 3K, 3S, 5; ITPBLL45	35.6%
III.1	20	FR1	AGC	ACC	ASPBLL2, 12, 13; BCPBLL8; LBPBLL4H; LKPBLL25	18.8%
III.1	52	CDR2	AGC	AAC	BCSynL56; BCPBLL6, 7, 9; ASPBLL10; LBPBLL4H; LKPBLL84, 85, 87, 88; ITPBLL25, 97	39.4%
III.1	87	FR3	TAC	TAT	ASSynL226; ASPBLL2; BCSynL56; BCPBLL6, 8; ITPBLL25	17.7%
III.1	94	CDR3	AGC	AAC	ASSynL227; ASPBLL10; BCPBLL4; LBPBLL4F, 4S; LKPBLL84; ITPBLL26	20.5%
6S1	43	FR2	TCC	GCC	BCSynL1, 3, 38, 59; BCPBLL16; LBPBLL2S, 4; ITPBLL75	88.9%

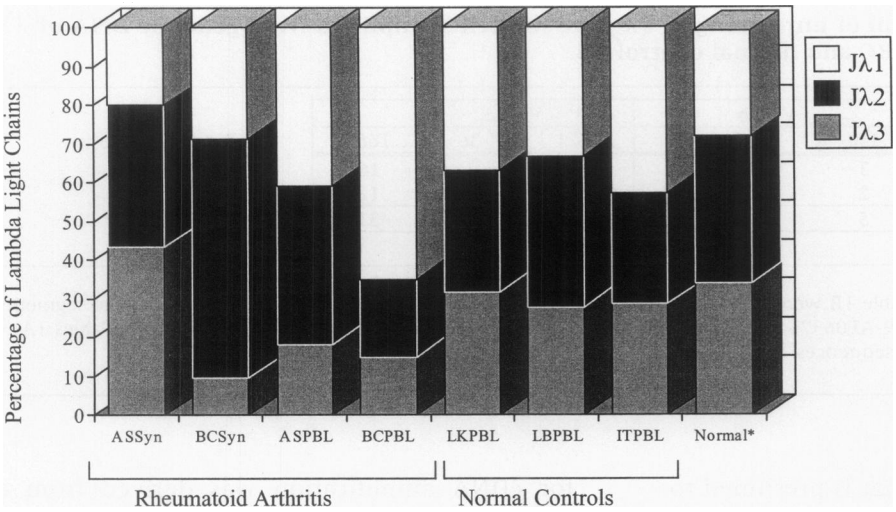

Fig. 4. Jλ gene segment utilization in RA synovia, RA PBL, and normal PBL. *, Utilization in lambda light chains from normal PBL (47).

three normal PBL samples (Jλ1 33–43%, Jλ2 29–39%, Jλ3 28–32%, Table 1C) and closely paralleled that seen in cDNA libraries from PBL of normal individuals (47) (Fig. 4). In RA tissues, however, there was more variability in Jλ gene segment utilization. For example, in synovium of patient AS, there was a slightly decreased representation of Jλ1 and an increased proportion of Jλ3, whereas AS PBL contained more Jλ1- and Jλ2-containing clones and fewer Jλ3-containing clones. Clones from BC synovium exhibited a marked increase in Jλ2 and a concomitant decrease in Jλ3, whereas BC PBL showed a marked increase in Jλ1.

Comparison of N-region Addition in Vλ and Vκ Transcripts from the Same Tissue Samples

The percentage of clones containing P nucleotides and N-region addition and the mean number of nucleotides added (among clones that contain N-region addition) are shown in Table 3. Lambda light chain data are shown in comparison to a similar analysis of predominantly VκIII transcripts from the same tissue samples [(16), Fig. 5]. P nu-

Table 3. N region and CDR3 lengths of immunoglobulin Vλ and Vκ clones from RA synovia and PBL and normal PBL

	Vλ					Vκ				
Tissue Sample	No. Clones Analyzed	P Addition	N Addition	Mean No. N's*	Mean CDR3 Length	No. Clones Analyzed	P Addition	N Addition	Mean No. N's	Mean CDR3 Length
AS Syn	30	13.3%	50.0%	3.3 ± 6.1	10.5 ± 1.4	11	27.2.%	54.5%	1.7 ± 1.2	9.8 ±1.0
AS PBL	20	30.0%	45.0%	2.4 ± 1.1	10.7 ± 1.3	10	20.0%	60.0%	1.7 ± 1.2	9.9 ± 0.9
BC Syn	21	33.3%	42.8%	1.9 ± 1.1	10.5 ± 1.1	14	35.7%	57.1%	1.8 ± 1.0	9.5 ± 1.2
BC PBL	20	10.0%	40.0%	3.0 ± 1.3	10.3 ± 1.0	15	33.3%	60.0%	1.9 ± 2.3	9.3 ± 1.0
LB PBL	18	16.7%	33.3%	2.5 ± 1.4	9.9 ± 1.1	12	25.0%	16.7%	3.0 ± 2.8	9.1 ± 0.5
LK PBL	19	15.8%	26.3%	2.6 ± 1.3	9.7 ± 1.6	11	18.1%	45.5%	3.8 ± 4.1	9.5 ± 0.7
IT PBL	28	32.1%	42.9%	2.2 ± 1.6	10.4 ± 1.1	11	18.1%	18.1%	2.0 ± 1.4	9.0 ± 0.6

Vκ data are derived from (16).

P addition and N addition refer to the percentage of clones containing at least one palindromic nucleotide or nucleotide of N-region addition, respectively. Mean number of nucleotides and CDR3 lengths (expressed as number of amino acid codons) are shown ± one standard deviation.

* The mean number of nucleotides refers to the average number of nucleotides added among the clones that contain N-region addition.

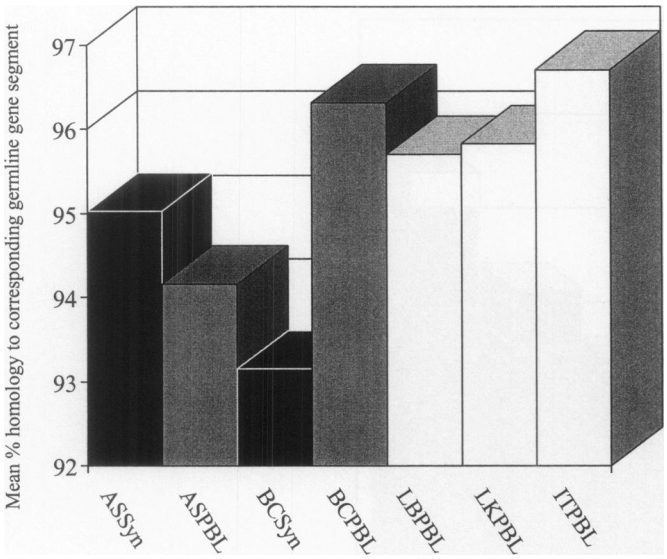

Fig. 5. Mean percent homology of expressed Vλ gene segments to corresponding germline gene segments. This analysis includes only clones that were mutated; clones with Vλ genes identical to germline were excluded. In general, clones from RA synovia (black bars) and RA PBL (gray bars) were more highly mutated (lower homology) than clones from normal PBL.

cleotides were present in 10–33% of Vλ transcripts and 18–36% of Vκ transcripts. There was no difference between proportions of Vλ clones with P nucleotides among RA tissues (10–33% of clones) and clones from normal PBL (16–32%). N-region addition in Vλ clones was surprisingly common in normal PBL (range 26–43%) but was more prevalent among clones from RA synovia (43–50%) and RA PBL (40–45%) (Fig. 6).

In all RA samples, there was more N addition in Vκ transcripts than in Vλ transcripts. Surprisingly, in two of the three normal individuals studied, N regions were more common among Vλ transcripts than in Vκ transcripts. Among clones with N-region addition the average number of nucleotides added ranged from 1.9 to 3.3 (Table 3). One of the AS synovial clones (ASSynL25) contained 25 nucleotides of junctional sequence, which may represent N-region addition or insertion of nucleotides through another mechanism (see Discussion). In both RA patients there was more N-region addition in Vλ transcripts from synovia than in those from PBL.

CDR3 Lengths of Lambda Light Chains from RA Synovia, RA PBL, and Normal PBL

CDR3 intervals were generally longer in Vλ transcripts (range 8–16 codons) than in Vκ transcripts from the same tissue samples (range

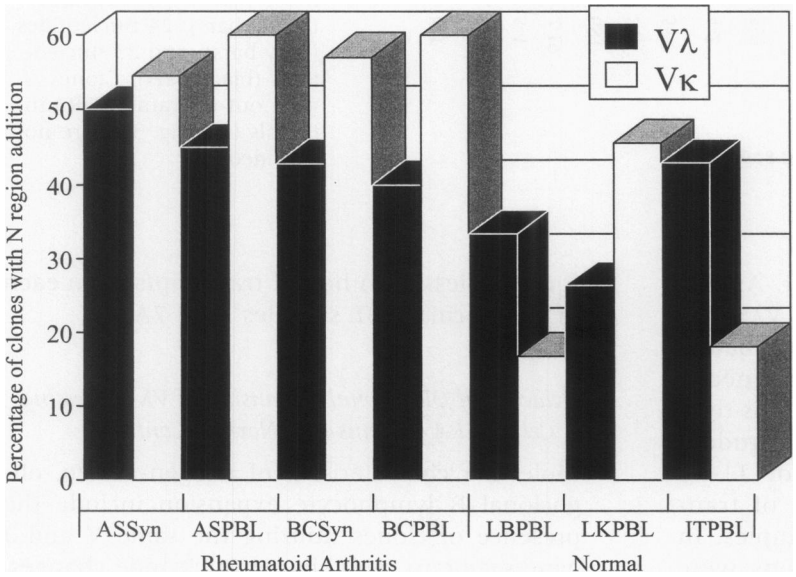

Fig. 6. N-region addition in Vλ-Jλ joins (black bars) and Vκ-Jκ joins (white bars) from RA synovia, RA PBL, and normal PBL. Vκ clones are from ref. 16 and derive primarily from members of the VκIII family. Percentages of Vκ clones containing N-region addition vary slightly from ref. 16 because of reanalysis to identify P nucleotides.

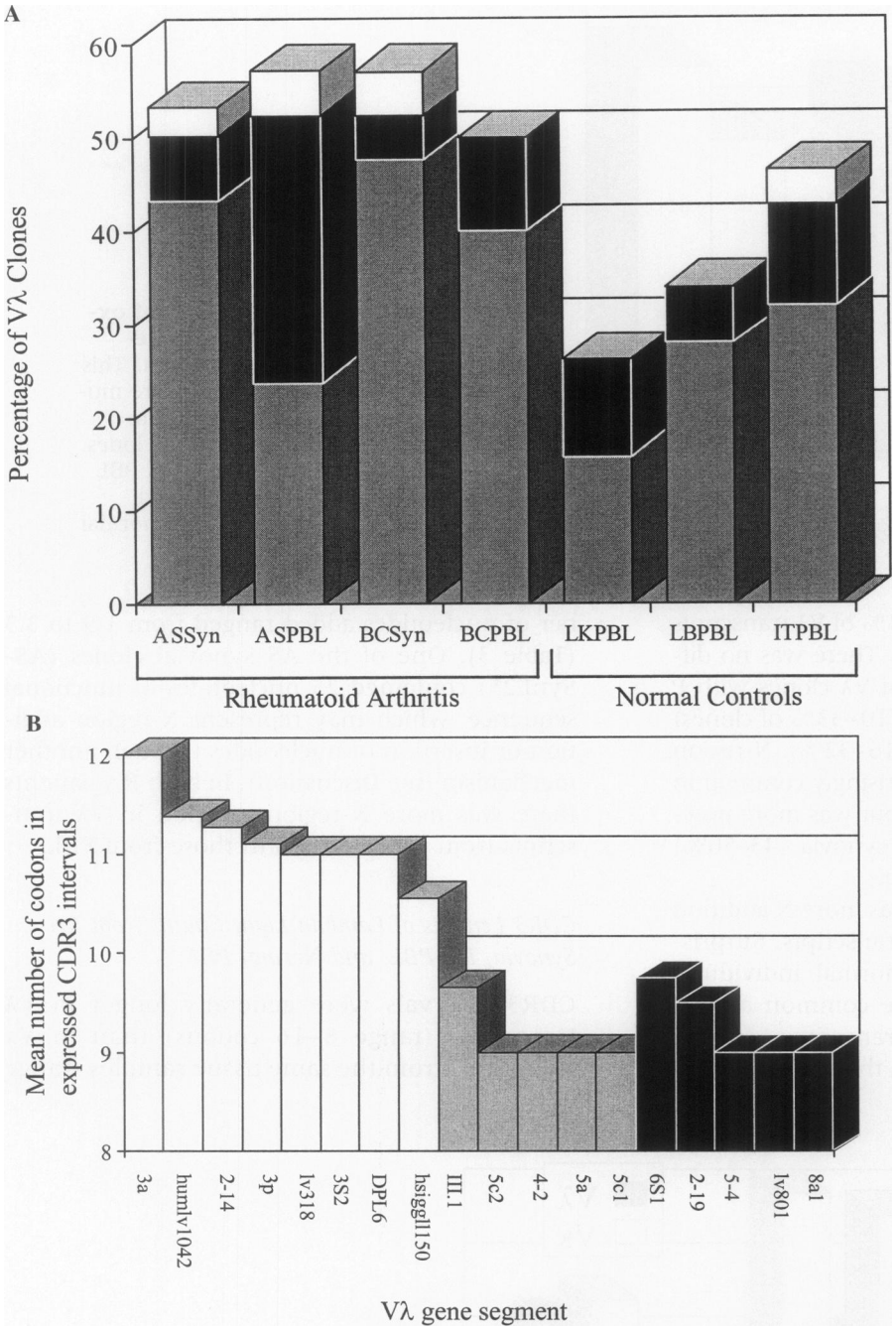

Fig. 7. (A) CDR3 lengths of Vλ clones from RA synovia, RA PBL, and normal PBL. The proportions of clones with CDR3 intervals of 11 codons (gray segments), 12 codons (black segments) and >12 codons (white bars) are shown. Proportions of clones with <10 codons are not represented. **(B)** Mean CDR3 lengths of clones according to the length of the germline component of the Vλ gene segment utilized. Germline gene segments are grouped into those with 29 nucleotides (white bars), 24 nucleotides (gray bars), and 23 nucleotides (black bars). Clones with out-of-frame CDR3 intervals (see Fig. 3) were not included.

7–11 codons) [(16), Table 3, Fig. 7A]. As was the case for kappa light chains (16), Vλ transcripts from RA synovium and PBL had a higher mean CDR3 length and contained a higher proportion of long CDR3 intervals than did those from normal PBL ($p = 0.02$, Student t-test, two-tailed). CDR3 intervals of 11 or more codons were found in ≥50% of transcripts from each of the four RA samples. In contrast, CDR3 lengths of ≥11 codons were found in less than half of transcripts from each of the normal PBL samples (Fig. 7A).

Evidence of Oligoclonal Expansion of Vλ-expressing B Cells in RA Patients and Normal Controls

Molecular characteristics of antigen-driven, oligoclonal B lymphocyte expansion include the presence of clones utilizing the same V and J gene segments with shared nucleotide changes,

similar N-region addition, and identical CDR3 lengths. Several sets of sequences that may represent the products of oligoclonal B-cell expansion were found among clones from RA patient AS synovium and PBL. Clones ASSynL182 and 189 are very mutated (92.3% and 92.3% homology to germline IGLV3S2) and have multiple shared differences (Fig. 8A). Both clones use the Jλ2 gene segment and have the same CDR3 length.

The Vλ gene segments from clones ASSynL211, 209, and 184 are derived from hsigll150 (96.6%, 91.6%, and 89.7% homology, respectively) (Fig. 8B). These three clones have identical Vλ-Jλ joins and CDR3 lengths. Importantly, clones ASSynL211 and 209 have a shared somatic mutation at codon 104 in the Jλ3 gene segment. Because all the germline Jλ gene segments are presumably known, this shared mutation is strong evidence of clonal relatedness. ASPBLL48 and 51 are also likely to be clonally related (Fig. 8C). They are heavily mutated in the Vλ gene segment (90.8% and 91.2% homology to hsiggll150, respectively), have identical Vλ-Jλ joins, and use the same heavily mutated Jλ1 gene segment. There are five nucleotide differences between ASPBLL48 and ASPBLL51, three of which lead to amino acid replacement. Patient BC also had evidence of clonal expansion in synovium and PBL. Clones BCSynL47 and 51 are heavily mutated and have shared differences at codons 28, 30, 77, and 93 (Fig. 8D); BCPBLL2 and 4 have shared changes at codons 50, 95A, and 96 (Fig. 8E). Among the clones from normal PBL, only individual LK demonstrated convincing evidence of clonal expansion. Clones LKPBLL76 and 72 are 96.2% and 95.0% homologous to hsiggll150, use the same Jλ gene segment, and have identical Vλ-Jλ joins (Fig. 8F). All but seven differences are shared between the two clones.

Levels of Somatic Hypermutation in RA Synovia and PBL and Normal PBL Transcripts Suggest the Subset of B Cells from Which They Are Derived

Vλ gene segments expressed in RA synovia were, on average, slightly more mutated than those from RA PBL, which were in turn more mutated than those from normal controls (Fig. 5). The mean percent homologies to germline nucleotide sequence were RA Syn 94.4%, RA PBL 95.4%, and normal PBL 96.7% ($p = 0.002$, ANOVA). Nine of 67 clones (14.9%) from normal individuals were unmutated, compared with only 3 of 94 (3.2%) of clones from RA samples ($p = 0.03$, Fisher exact, two-tailed). When unmutated sequences were omitted from the analysis, the differences in amount of somatic mutation were still apparent (RA Syn 94.2%, RA PBL 95.2%, and normal PBL 96.1%, $p = 0.02$, ANOVA).

In this cDNA analysis, quantitative differences in immunoglobulin mRNA expression among B cells at various stages of differentiation probably explain discrepant levels of somatic mutation between RA synovia and RA PBL. Synovial infiltrates from patients with long-standing RA typically contain many plasma cells (54,55), which have been shown to have high immunoglobulin mRNA levels (56). Class-switched and IgM^+/IgD^- memory B cells have also been reported to express higher levels of immunoglobulin mRNA than naive B cells (57). Marked levels of somatic mutation were noted in our previous analyses of the synovial IgG V_H repertoire of RA patient BC (mean homology to germline 91%) (58) and the synovial Vκ repertoires of RA patients BC (mean homology 94.9%) and AS (mean homology 95.8%) (16). Thus, the increased somatic mutation in lambda light chains from RA synovia is likely attributable to the presence of plasma cells, which express high levels of mutated sequences. There were fewer unmutated sequences from RA PBL than from normal PBL. Thus, it seems likely that more RA PBL sequences originated from memory B cells (either class-switched or IgM^+/IgD^- memory B cells) than from the essentially unmutated naive B cell (IgM^+/IgD^+) population. In contrast, more of the normal PBL sequences may have been derived from naive B cells.

Potential PCR Artifacts

We used sequence comparison software that is capable of comparing individual FR and CDR domains with corresponding domains from multiple germline gene segments. Thus, we were able to identify sequences with crossover events involving two different gene segments. We found only one such crossover artifact. BCSynL32 is a clone that contains a crossover artifact, with the FR1 and CDR1 domains from the VλVI gene segment 1v6S1 and FR2, CDR2, FR3, and CDR3 from the VλI gene segment humlv1042 (DPL8) (Fig. 2). This so-called jumping PCR artifact occurs when an incomplete product of one amplification cycle serves as a primer for a related sequence, generating a chimeric molecule (59–61).

A

	FR 1					CDR 1								FR 2						
Codon	4	5	12	17	20	26	27	28	29	30	31	32	33	37	38	39	45	46	48	49
IGLV3S2	CTG	ACT	TCA	AAG	AGG	AAC	AAC	ATT	GGA	AGT	AAA	AGT	GTG	CAG	CAG	AAG	GTG	CTG	ATC	TAT
ASSynL182	G..	...	..G	...	.C.	G..	...	...	.A.	...	CT.	...	...	...	...	.G.	C..	...	..A	C..
ASSynL189	...	C..	...	...	...	...	...	...	...	G	T.C	...	..C	..A	A..	...	...	.A.	...	..C

	CDR 2	FR 3					CDR 3						Jλ2		
Codon	50	57	66	68	76	79	90	92	93	94	95B		96	97	107
IGLV3S2	TAT	GGG	AAC	GGG	AGC	GAA	GTG	GAC	AGT	AGT	CAT	CC	T GTG	GTA	CTA
ASSynL182	.C.	..A	..T	..C	.CT	..G	..A	..T	.T.	.C.	.C.		...	A..	...
ASSynL189	.G.	...	...	...	...	...	...	..G	...	...	...		...	...	...

B

	FR 1					CDR 1					FR 2								CDR 2				
Codon	1	3	14	18	20	27	29	30	32	34	36	37	38	40	43	46	48	49	50	51	52	53	54
hsiggl1150	TCC	GAG	TCC	ACG	AGG	GCA	CCA	AAG	TAT	TAT	TAC	CAG	CAG	CCA	GCC	CTG	ATA	TAT	AAA	GAC	AGT	GAG	AGG
ASSynL211	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ASSynL209	...	...	...	...	...	...	T..	...	.T.	...	..T	...	..A	...	...	...	..G	...	TT.	A..	...	A..	...
ASSynL184	..T	..A	.A.	T..	.CT	..G	...	.T.	...	...	...	...	...	G..	..T	AC.	..C	.T.	G.G	...	...	...	C..

	FR 3										CDR 3						N Region	Jλ3					
Codon	60	64	65	66	71	72	77	80	81	87	89	93	94	95	95A	95B		96	97	103	104	105	106	107
hsiggl1150	GAG	GGC	TCC	AGC	GTC	ACG	GGA	GCA	GAA	TAC	CAA	AGC	AGT	GGT	ACT	TAT	CC	T TGG	GTG	AAG	CTG	ACC	GTC	CTA
ASSynL211	..A	...	...	...	...	...	...	...	.G.	..T	...	..A	...	..G	...	...	.	. ...	...	...	...	...	..T	...
ASSynL209	..A	..T	..G	..T	...	...	...	T..	.G.	..T	...	...	...	...	...	...	.	. ...	...	...	...	...	...	..G
ASSynL184	...	...	...	GC.	..T	..A	.AG	...	...	..T	..G	..A	CT.	...	...	...	.	. ..C	C.C	...	..T	...	T..	

C

	FR 1				CDR 1				FR 2				CDR 2		
Codon	3	7	12	19	25	31	32	33	37	46	48	49	52	54	56
hsiggl1150	GAG	CCA	TCA	GCC	GGA	CAA	TAT	GCT	CAG	CTG	ATA	TAT	AGT	AGG	TCA
ASPBLL51	..	..	..	..	..	..	.T	..	..	T..	..	..	..	..	..
ASPBLL48	..	..	..	..	..	.T	.T	..	..	T..	..	..	..	..	..

	FR 3				CDR 3								Jλ1					
Codon	66	72	76	85	89	90	93	94	95	95A	95B		96	97	100	102	103	105	107
hsiggl1150	AGC	ACG	AGT	GAC	CAA	TCA	AGC	AGT	GGT	ACT	TAT	CC	T TAT	GTC	ACT	ACC	AAG	ACC	CTA
ASPBLL51	..	..	..	...	..	..	..	..	...	..	...		...	...	..	..	..	...	.G
ASPBLL48	..	..	..	..G	..	..	..	..	..A	..	...		...	...	..	..	..	T..	..

D

	FR 1								CDR 1									
Codon	2	3	4	5	11	13	20	22	24	25	27	28	29	30	31	32	33	34
hsiggl1150	TAT	GAG	CTG	ACA	GTG	GTG	AGG	ACC	TCT	GGA	GCA	TTG	CCA	AAG	CAA	TAT	GCT	TAT
BCSynL47	.C.	...	T..	..G	...	A..	.C.	...	...	...	C..	...	T..	TT	...	.T.	...	...
BCSynL51	...	..A	...	...	A..	...	...	.A.	..C	A..	..G	.C.	.T.	G.	GC.	...	TT.	...

```
                                FR 2                                    CDR 2
                 36  37  38  40  41  45  46  47  48  49    50  51  52  53  55
hsiggll150       TAC CAG CAG CCA GGC GTG CTG GTG ATA TAT   AAA GAC AGT GAG CCC
BCSynL47         ... ..A A.. ... ..G C.C T.. ... ..G .C.   ... ... GA. ... ..G
BCSynL51         ..T ... ... G.. ... ... A.. A.A ..T .T.   ..G A.. .T. .G. ...

                                                FR 3
                 59  60  62  64  66  67  69  70  71  72  74  75  76  77  80  81  85  86  87
hsiggll150       CCT GAG TTC GGC AGC TCA ACA ACA GTC ACG ACC ATC AGT GGA GCA GAA GAC TAT TAC
BCSynL47         T.. ... ..G ... ..G ..C G.C ... ... .AC .T. ..T ... .▌. ..G ..G ..A ... ...
BCSynL51         ... ..A A.. .CT ... ... ... .AT TC. ..T ... ... .A. A▌C ... ... ... .T. ..T

                                CDR 3                                       Jλ2
                 88  89  90  91  92  93  94  95 95A 95B     96  97   98 103 104 107
hsiggll150       CAA TCA GCA GAC AGC AGT GGT ACT TAT CC   T GTG GTA  TTC AAG CTG CTA
BCSynL47         ... ... ... ... .▌G ..G ..C ..G A▌                ...  ..T ... T.. ..G
BCSynL51         ..G ... A.. ... .▌T .C. ..G .A. .▌                C.T  ... ..A ... ..C
```

E

```
                FR 1      CDR 1      CDR 2      CDR 3                Jλ1
                  4    28  30  34    50    93  94  95 95A      96  97
III.1           CTG   TTG GAT TGC   CAA   AGC AGC ACT GCA   T TAT GTC
BCPBLL2         ...   ... ... ...   ▌..   ... ... ... ▌.    . .▌▌ ...
BCPBLL4         G..   C.. A.. .A.   ▌..   .A. .A. ... ▌.    . .▌▌ ...
```

F

```
                FR 1   CDR 1          FR 2              CDR 2     FR 3          CDR 3          P          Jλ1
                  3    30  33    40  46  48  49    50  52    66  69    91  95 95A 95B          96  97  98
hsiggll150      GAG   AAG GCT   CCA CTG ATA TAT   AAA AGT   AGC ACA   GCA GGT ACT TAT       T TAT GTC TTC
LKPBLL76        ▌..   .▌. ...   ▌.G ▌.. ..▌ ...   ▌. .C.   .▌. ...   ... ... .          ▌      . ... ...
LKPBLL72        ▌..   .▌C A..   ▌.. ▌.. ..▌ .T.   ▌. .C.   .▌. ..G   C.. ... .          ▌      . ... .C.
```

Fig. 8. Nucleotide sequences that may be the result of oligoclonal B-cell expansion, defined as utilizing the same V and J gene segments with shared mutations and having identical CDR3 lengths. Only codons for which there are differences from the corresponding germline gene segment are shown. Dots denote nucleotide homology. Shared nucleotide changes are shown as shaded boxes, with changes that may represent possible allelic variations (see Table 2) underlined. Palindromic (P) nucleotides are shown in lower case. (A) IGLV3S2-derived clones from synovium of RA patient AS. (B) hsiggll150-derived clones from synovium of RA patient AS. (C) hsiggll150-derived clones from PBL of RA patient AS. (D) hsiggll150-derived clones from synovium of RA patient BC. (E) VλIII.1-derived clones from PBL of RA patient BC. (F) hsiggll150-derived clones from the PBL of normal individual LK.

Although our sequence analysis software can easily identify a hybrid molecule containing two different germline Vλ gene segments, identification of crossovers between two transcripts expressing the same Vλ gene segment represents a more difficult problem. In all clones thought to be genealogically related (Fig. 8), there were nonshared differences in multiple FR and CDR domains. To explain these findings by jumping PCR artifact, one would have to posit that multiple, closely spaced crossover occurred. This is unlikely from a statistical point of view because of the relatively small number of PCR cycles used for amplification.

Because a nonproofreading DNA polymerase (*Taq* DNA polymerase) was used in PCR, we were concerned about the possibility of PCR errors. Estimates of the fidelity of *Taq* DNA polymerase vary from 2.0×10^{-5} errors per base pair (62) to 1.1×10^{-4} errors per base pair (63,64); 2.4×10^{-5} frameshift mutations per base pair are estimated to occur in *Taq* DNA polymerase PCR reactions (63). The sequence analysis of unrearranged Vλ gene segments from T lymphocytes, in which somatic hypermutation of immunoglobulin genes does not occur, allowed us to estimate the *Taq* error rate. From a total of 9472 base pairs that were sequenced, five single base pair substitutions (and no frameshift mutations) were found. If one assumes that all five of these nucleotide changes were due to *Taq* error, the maximum error rate is 5.3×10^{-4} error changes per base pair. Thus, in the reported cDNA sequences, the error rate is presumably very low (approximately one base pair error in every third sequence).

Discussion

N-region Addition during Immunoglobulin Lambda Light Chain Gene Rearrangement

The finding of N-region addition in Vλ-Jλ joins from RA patients and normal individuals has important implications regarding the generation of CDR3 diversity during immunoglobulin gene rearrangement in normal B cells. In human bone marrow, stem cells progress through the pro-B-cell, pre-B-cell, and immature B-cell stages of differentiation before becoming mature B cells (reviewed in ref. 65). In the pro-B-cell stage, the immunoglobulin heavy chain locus undergoes rearrangement and the enzyme responsible for N-region addition, TdT, is present in the nucleus of the cell. In the pre-B-cell stage, kappa light chains undergo rearrangement. Although TdT protein has not been shown to be present at the time of kappa light chain rearrangement, N-region addition is clearly present in kappa light chains from normal individuals and those with RA (15–18).

The current theory for immunoglobulin light chain rearrangement holds that if successful kappa rearrangement fails on both alleles, then lambda light chain rearrangement occurs. Thus, lambda light chain rearrangement is thought to occur well after the expression of TdT. Our analysis of Vλ-Jλ joins, however, provides clear evidence of N-region addition, presumably due to TdT activity at the time of lambda light chain rearrangement. Surprisingly, in two of the three normal individuals studied, there was N-region addition in a higher proportion of Vλ transcripts than Vκ transcripts. Thus, our data provide support for the hypothesis that TdT is present throughout all stages of immunoglobulin gene rearrangements in B cells from normal individuals. We also found more N-region addition in lambda light chains from patients with RA than in those from normal individuals. The same is true of the kappa repertoire of RA patients (Fig. 6) (16), which suggests that TdT may be abnormally regulated in RA.

There are several potential explanations for the presence of non-germline-encoded nucleotides at the V-J junctions of lambda light chains. One possibility is that lambda light chain rearrangement occurs earlier than was previously thought, such as before heavy chain rearrangement or after heavy chain rearrangement but before kappa light chain rearrangement. Kubagawa et al. documented pro-B-cell lines that had undergone kappa rearrangements at one or both alleles but contained heavy chain loci without V_H-D_H-J_H rearrangements (66). Some of the kappa light chain rearrangements in these kappa-only cell lines do contain N regions (67).

An alternative explanation for N-region addition in λ chains is that the current theory of rearrangement order (heavy chain, then kappa light chain, then lambda light chain) is correct, but that TdT is expressed in B cells through the mature B cells stage. Support for this finding lies in the description of a small subpopulation of pre-B cells expressing both nuclear TdT and cytoplasmic μ heavy chains (68).

Allelic Variations in the Human Immunoglobulin Lambda Locus

In addition to providing another mechanism for antibody diversity, the presence of allelic variants in the human lambda locus has important implications regarding the analyses of somatic hypermutation and assessment of clonality. The amounts of somatic hypermutation in this study were calculated with the assumption that clones were derived from known Vλ gene segments. Each expressed Vλ gene segment was assigned a presumed progenitor gene segment based on highest degree of homology. Although the human Vλ locus has been sequenced by two groups of investigators (20,21), there may be unreported variants of Vλ gene segments. The levels of somatic mutation and the degree of oligoclonality reported here may be overestimated if there are Vλ gene segments or additional unreported allelic variations of previously described germline gene segments.

Kawasaki et al. assessed the degree of allelic variation of the human immunoglobulin lambda locus by comparing nucleotide differences between their sequence and two cosmid clones from different sources (one from a Caucasian individual and another from a Japanese individual) (21). They found 100 nucleotides over a span of 39,236 nucleotides that were thought to represent allelic variations. Although the number of differences located within coding regions was not reported, there appear to be relatively frequent single nucleotide variations in the human immunoglobulin lambda locus. Thus, additional novel allelic variants are likely to be found in future studies of lambda light chain expression.

Nucleotide Insertions and Deletions in Vλ Gene Segments May Occur during Somatic Hypermutation in a Germinal Center (GC) Reaction

Single or multiple nucleotide insertions and deletions have been observed in heavy chain variable (V_H) domains from germinal center (GC) B cells. Using a single-cell PCR approach, Goossens et al. sequenced rearranged V_H domains from naive and GC B cells (69). No deletions or insertions were found in naive B cells, but ~4% of in-frame and >40% of out-of-frame (OOF) rearrangements of GC B cells had deletions and/or insertions of variable length. Wilson et al. also noted insertions and deletions in somatically mutated V_H gene segments from $CD38^+/IgD^-$ GC B cells and $CD38^-/IgD^-$ memory B cells from human tonsil (70). Eight of nine insertions/deletions were present in the CDR1 or CDR2 domains. In our previous analysis of 18 immunoglobulin V_H gene segments from RA synovium, we found one highly mutated V_H4-containing transcript with a two-codon insertion in CDR1 (71).

In the present study, we found two lambda light chain clones with insertions or deletions that may be the result of somatic hypermutation in the GC reaction. ASSynL191 has a one-codon deletion in CDR1 and LKPBLL68 contains a one-codon insertion in CDR1 (Fig. 2). Both clones are somatically mutated: ASSynL191 is 95.2% homologous to its progenitor germline gene segment, while LKPBLL68 shares 92.3% homology to its germline gene segment. Several clones in the present study were found to have single base pair insertions or deletions that may reflect somatic mutation (69) or alternatively, frameshift artifacts induced by *Taq* DNA polymerase. Our data suggest that insertions and deletions occur as a result of somatic hypermutation not only in heavy chains but in lambda light chains as well.

CDR3 Intervals of Lambda Light Chains Are Longer than Those of Kappa Light Chains and the Lengths Are Heavily Influenced by Vλ Gene Segment Utilization

In both RA patients and normal individuals, lambda CDR3 intervals were longer, on average, than CDR3 intervals from the kappa light chains of the same tissue samples. In immunoglobulin light chain rearrangements, the number of nucleotides at the V_L-J_L join and thus the CDR3 length are dependent upon the amount of exonuclease activity at the V and J termini of the join and the number of N nucleotides inserted by TdT. Germline Jκ and Jλ gene segments typically contain 7 nucleotides in the region that encodes the CDR3 (16). Vκ gene segments have CDR3 components that are remarkably constant in length (23 nucleotides). In contrast to Vκ gene segments, there is considerable variation in the number of nucleotides in the CDR3 components of germline Vλ gene segments. Gene segments IGLV3S2, 2-14, humlv318, hsiggll150, 3p, 3a, humlv1042, and DPL6 contain 29 nucleotides in the germline CDR3 component. Gene segments III.1, 4-2, hsiglv5a, hsiglv5c1, and hsiglv5c2 have 24 nu-

cleotides in the CDR3 component, while gene segments 2-19, 8A1, lv801, 6s1, and 4a have 23 nucleotides.

In the present study, the 96 clones containing Vλ gene segments with 29 nucleotides in the germline CDR3 component had a mean CDR3 length of 10.9 codons, the 41 clones containing Vλ gene segments with germline CDR3 component of 24 nucleotides had a mean CDR3 length of 9.5 codons, and the 16 clones with Vλ gene segments containing 23 nucleotides had a mean CDR3 length of 9.4 codons ($p < 0.0001$, ANOVA) (Fig. 7B). Thus, in contrast to the situation in the kappa repertoire, the germline Vλ gene segment used in the rearrangement has a strong influence on the length of the expressed cDNA CDR3 interval.

Enrichment for Unusually Long CDR3 Intervals in Both Lambda and Kappa Light Chains in RA Patients

Compared with normal PBL, RA synovia and PBL had higher proportions of clones with long CDR3 intervals. This difference is explained in part by a higher proportion of Vλ gene segments with long CDR3 components among the RA clones. Gene segments IGLV3S2, 2-14, humlv318, hsiggll150, 3p, 3a, humlv1042, and DPL6, which contain 29 nucleotides in the germline CDR3 component were found in 64.8% of RA clones, versus 57.6% of normal controls. Another contributing factor to enrichment of longer CDR3 intervals in RA is a higher proportion of clones with N-region addition.

The enrichment of longer CDR3 intervals in both the kappa and lambda light chain repertoires of RA patients suggests either positive antigenic selection of unusual B cells or abnormal immunoglobulin gene rearrangement. These explanations are not mutually exclusive, and both may occur to some degree in RA patients. In the first scenario, lambda light chains expressing unusually long CDR3 intervals are generated in both normal individuals and RA patients. Subsequently, normal individuals show no preferential positive selection of B cells that by chance contain kappa or lambda light chains with unusual CDR3 intervals. In contrast, RA patients exhibit positive antigenic selection and clonal outgrowth of such B cells, possibly as a result of the chronic inflammatory response. Support for this hypothesis is the finding that alterations in the sequence at Vκ-Jκ joins can abrogate antigen binding of the antibody (72).

In addition, there is substantial evidence that RA is an antigen-driven disease (17,24,73–77). We have previously reported oligoclonal B-cell expansion in the immunoglobulin heavy chain (58,78) and kappa light chain (27) repertoires of synovial tissue of RA patient BC, which suggests an antigen-driven B-cell response. Furthermore, the clonally expanded kappa light chain clone contained an unusually long CDR3 interval (27). In the present study, we found sets of genealogically related sequences in the lambda repertoire of RA patients BC and AS. Oligoclonality of the synovial B-cell population thus appears to be a common feature of RA synovium.

An alternative explanation for the unusual Vλ-Jλ joins in RA is that there may be differences in the regulation of expression of proteins involved in the rearrangement process, such as RAG-1, RAG-2, and TdT, resulting in abnormal development of the naive B-cell repertoire in bone marrow. Immunoglobulin heavy and light chain gene segments undergo a highly regulated series of V(D)J gene segment rearrangements to generate a functional antibody. V(D)J recombination requires a RSS (composed of a heptamer, 12- or 23-base pair spacer and a nonamer), RAG-1, and RAG-2 (79). Formation of a double-strand break at a RSS occurs during V(D)J recombination, with the cleavage reaction divided into two distinct steps: nicking and hairpin formation. A nick is introduced at the 5′-end of the signal sequence, followed by breakage of the other DNA strand, resulting in a hairpin structure at the coding end and a blunt signal end (79). A DNA-binding protein complex containing Ku70, Ku80 subunits, RAG-1, RAG-2, and DNA ligase performs V(D)J joining (80).

Clone ASSynL25 is of particular interest with regard to possible dysregulation of immunoglobulin gene segment rearrangement in RA. The Vλ-Jλ join of this clone contains 25 nucleotides that do not appear to be encoded by the VλIII.1 or Jλ2 sequence, resulting in a CDR3 interval of 16 amino acid codons (Fig. 3). One possible explanation for this extremely long Vλ-Jλ join is N-region addition, but a more likely explanation for the presence of this 25 base pair insertion is the presence of an insertion that is duplicated from the VλIII.1 gene segment. Sixteen of the 25 nucleotides at the Vλ-Jλ join are identical to the sequence in codons 90 to 95A of the germline

III.1 gene segment, shown as underlined nucleotides in Figure 3A. Thus, there is evidence of unusual immunoglobulin rearrangement in RA synovium.

Expression of RAG-1 and RAG-2 and Receptor Editing in B Cells in Normal Secondary Lymphoid Organs: Implications for RA and Other Chronic Inflammatory Diseases

Among the mechanisms of avoiding production of autoreactive sequences are clonal deletion, clonal anergy, and receptor editing. Receptor editing involves changing an autoreactive variable region to a different (presumably nonautoreactive) variable region through a secondary rearrangement (81–83). In order for this to occur, the V(D)J recombination machinery must be maintained or reactivated. RAG-1 and RAG-2 were initially thought to be expressed in B cells only during B-cell development in bone marrow. However, RAG-1 and RAG-2 have recently been found to be expressed in germinal center (GC) B cells of mice, with evidence of secondary rearrangements and receptor editing (84–86).

There is circumstantial evidence suggesting that RA synovia may act as a lymphoid organ. Nodular lymphocytic infiltrates that are histologically similar to normal GC have been found in the subsynovial layer of some patients with RA (87–90), Lyme disease (91), and reactive arthritis (92), and in inflamed liver tissue from patients with chronic hepatitis B and C (93–95). We have documented the presence of clonally related immunoglobulin heavy and kappa light chain sequences in RA synovium, which suggests in situ antigenic selection (27,58,78). Berek and colleagues have reported clonally related sequences from within GC-like structures in RA synovia (77). The histologic similarities between RA synovia and normal lymphoid tissues containing GC, and the recent findings of RAG-1 and RAG-2 expression in a subset of normal GC B cells suggest that RAG-1 and RAG-2 may be expressed in RA synovia. If so, secondary rearrangements may be possible in synovium of patients with long-standing RA.

In summary, these data demonstrate that in humans, N-region addition enhances antibody diversity at all stages of immunoglobulin gene rearrangement. The structural diversity of lambda light chain CDR3 intervals is greater than that of kappa light chains, largely due to variability in the lengths of germline Vλ gene segments. As in the kappa light chain repertoire, the lambda light chains of RA patients are enriched for sequences with long CDR3 intervals. Finally, the presence of clonally related sequences in RA synovia and PBL supports an antigen-driven B-cell response in RA. We speculate that RAG-1 and RAG-2 expression may occur in a subset of RA synovial B cells, leading to secondary immunoglobulin gene rearrangements, as has recently been reported in normal peripheral lymphoid organs. The presence of GC-like structures capable of generating a B-cell response within RA synovia raises the possibility that local antigens can propagate the chronic inflammatory response.

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