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Templated Nucleotide Addition and Immunoglobulin J_H-Gene Utilization in t(11;14) Junctions: Implications for the Mechanism of Translocation and the Origin of Mantle Cell Lymphoma[1]

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ABSTRACT

The t(11;14)(q13;q32) between the *BCL-1* and immunoglobulin heavy chain gene (*IgH*) loci in mantle cell lymphoma (MCL) are believed to be mediated by the mechanism of V(D)J recombination similar to the t(14;18) in follicular lymphoma (FL). We have recently shown that the t(14;18) event creates staggered double-strand breaks in the *BCL-2* locus, and that the t(14;18) junctions contain templated nucleotide insertions (T-nucleotides; U. Jäger *et al.*, Blood, *95:* 3520–3529, 2000). Reasoning that the earlier (pregerminal center) B-cell origin of MCL might be reflected in a different molecular structure of the chromosomal breakpoints, we PCR-amplified diagnostic samples from 93 patients. Thirty-six samples (39%) were positive for the direct (*BCL-1*/J_H) and 23 for both direct and reciprocal (D_H/*BCL-1*) junctions. The breaks on chromosome 14 exhibited features of V(D)J-mediated recombination as shown by D_H and J_H coding end processing. However, duplications of *BCL-1* sequences in 39% of the 23 patients indicate staggered double-strand breaks in the major translocation cluster region (MTC). This is incompatible with V(D)J recombination and indicates a different mechanism of cleavage. The use of J_H6 in the junctions (39%) was similar to that in the immunoglobulin genes of normal B cells and B-CLL, but considerably less than in FL. Only 2 of 36 samples contained a *BCL-1*/DJ_H rearrangement, which was indicative of a previous DJ_H rearrangement. Most importantly, 19% of the *BCL-1*/*IgH* junctions with inserts of ≥5 nucleotides contained error-prone copies (T-nucleotides) of 8–12 nucleotides originating from the surrounding *BCL-1* or *IgH* regions, a lower rate than in FL. No correlation was found between the addition of T-nucleotides and the rate of somatic mutation in the immunoglobulin genes. We conclude that the t(11;14) and t(14;18) use the same basic mechanism of translocation including V(D)J-mediated recombination, double-strand staggered breaks, and template-dependent, error-prone DNA-synthesis. However, the distinct differences in the utilization of J_H regions suggest that the t(11;14) occurs predominantly during an attempted primary D_H-J_H rearrangement in early B cells, whereas the t(14;18) mostly occurs during secondary rearrangement. This is in agreement with the pregerminal center B-cell origin of MCL.

INTRODUCTION

MCL[3] is a non-Hodgkin's lymphoma arising from a subset of naive B cells localized in primary follicles or in the mantle region of secondary follicles (1, 2). The lymphoma cells are characterized by a CD19+, CD5+, CD23− phenotype. The majority of MCLs show little or no somatic mutation of their immunoglobulin genes, which suggests that they originate from pregerminal center B cells (3). However, some cases exhibit signs of ongoing mutations, which suggests that MCLs represent a heterogeneous entity (4, 5). The specific hallmark of MCL is overexpression of the cyclin D1 protein caused by the chromosomal translocation t(11;14)(q13;q32) that juxtaposes the *BCL-1* (*PRAD-1*) gene with the immunoglobulin heavy chain locus (6–8). Thpp t(11;14) can be detected by fluorescence *in situ* hybridization in virtually all patients (9–12). At the molecular level, the translocation is reciprocal, creating a *BCL-1*/J_H fusion on the der14 chromosome and a D_H/*BCL-1* fusion on the der11 chromosome (13). Thirty to 55% of the breaks in *BCL-1* occur within the 80- to 100-bp MTC and can be PCR-amplified (Refs. 14–18; Fig. 1).

Recent evidence suggests that the process of translocation may be preceded by alterations affecting the genomic stability, such as deletions or mutations in the *ATM* gene (19–23). The t(11;14) event itself is believed to occur at the time of an attempted D_H-J_H rearrangement in early B cells. The pattern of direct (*BCL-1*/J_H) and reciprocal (D_H/*BCL-1*) junctions as well as the presence of presumably nontemplated nucleotides ("N-regions") added by Tdt suggested that the mechanism of V(D)J recombination is responsible for this illegitimate joining similar to the t(14;18) translocation in FL (24–28). Some authors proposed the presence of cryptic immunoglobulins RSSs in the *BCL-1* MTC (25). However, these hypotheses are based on only a few observations in cloned or PCR-amplified DNA sequences.

We have recently shown that the t(14;18) in FL involves at least two distinct mechanisms: V(D)J recombination mediating the breaks on chromosome 14, and an additional mechanism, distinct from V(D)J recombination and yet unidentified, creating the breaks on chromosome 18. In addition, this analysis revealed the insertion of T-nucleotides in the junctions between the *BCL-2* and J_H or D_H genes (29, 30). T-nucleotides are copied from the regions surrounding the breakpoints and contain point mutations and/or insertions/deletions suggesting the presence of error-prone template-dependent DNA synthesis at the time of illegitimate joining. Moreover, in the t(14;18), we found a marked skew toward the most 5′-D_H and most 3′-J_H regions, which suggested that the t(14;18) in FL could occur during an attempted secondary rearrangement.

This prompted us to investigate the direct and reciprocal t(11;14) junctions in a large sampling of MCL. This should enable us to compare the t(14;18) and t(11;14) and to test the hypothesis that they are generated by a similar mechanism of translocation despite their different cellular origin: germinal (FL) *versus* pregerminal center (MCL). We analyzed diagnostic material from 93 MCLs by PCR and

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[3] The abbreviations used are: MCL, mantle cell lymphoma; MTC, major translocation cluster (region); ATM, ataxia teleangiectasia mutated; Tdt, terminal deoxynucleotidyl transferase; FL, follicular lymphoma; RSS, recombination signal sequence; T-nucleotide, templated nucleotide; B-CLL, B-cell chronic lymphocytic leukemia; SHM, somatic hypermutation mechanism.

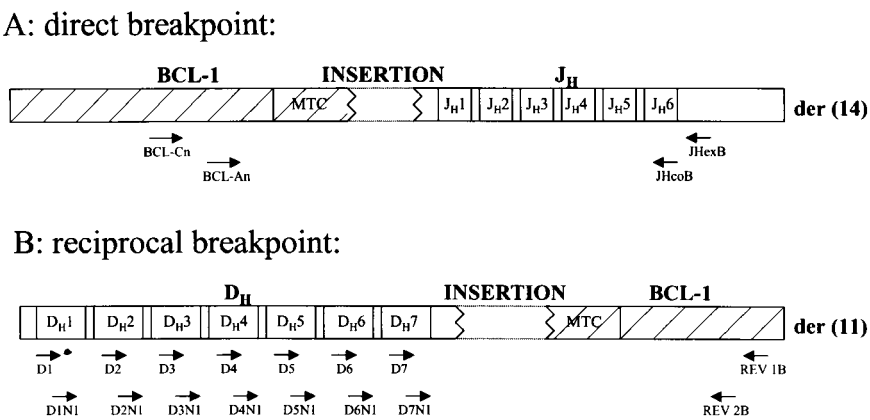

Fig. 1. Primers used for the amplification of (*A*) *BCL-1*/J_H (direct) and (*B*) D_H/*BCL-1* (reciprocal) breakpoints. *Hatched box*, the *BCL-1* gene, including the MTC-region; *broken lines* (*INSERTION*), *de novo* insertions; *numbered*, D_H/J_H families; *arrows*, the different primers.

obtained sequence information on 36 *BCL-1*/J_H (direct) junctions (39%). These 36 samples were further amplified with primers for the D_H/*BCL-1* (reciprocal) junctions giving a final number of 23 direct as well as reciprocal positive samples. Analysis of this library revealed that the t(11;14) uses the same mechanism as t(14;18) involving RSS-mediated breaks at the IgH locus, but a different mechanism of cleavage at the oncogene breakpoint region. MCLs also show the presence of T-nucleotides in their t(11;14) junctions. However, the number of T-nucleotides as well as the usage of J_H segments show distinct differences from FL which are consistent with the earlier B-cell origin of MCL.

MATERIALS AND METHODS

Patients and DNA Samples. We analyzed samples from 93 patients diagnosed to have MCL according to the Revised European-American Lymphoma classification after informed consent was obtained (1). DNA was prepared from various diagnostic tissues including peripheral blood ($n = 16$), bone marrow ($n = 27$), and fresh ($n = 7$) or paraffin-embedded ($n = 43$) lymph nodes, according to standard procedures. Two different materials were analyzed from four direct and reciprocal PCR-positive patients to confirm intrasample fidelity.

PCR Amplification of the Direct and Reciprocal Breakpoints. Genomic DNA (100 ng) was amplified for the direct (*BCL-1*/J_H) breakpoints with BCL-Cn and JHex-B (29) primers (Fig. 1) for 30 cycles with the following conditions: 1 min at 94°C and 1 min at 58°C. The BCL-Cn primer is located 184 bp 3′ of the *BCL-1* sequence (GenBank accession no. S77049). One μl of the primary PCR was used for secondary nested amplifications with BCL-An and JHco-B (29) primers for 25 cycles with the same conditions as above except for the annealing temperature, 61°C.

For the reciprocal breakpoints (D_H/*BCL-1*) a set of seven D_H primers (29) mapping all of the members of each family was used. Again, 100 ng of genomic DNA were amplified with one of the D1 to D7 primers and the REV 1B primer for 30 cycles (30 s at 94°C, 30 s at 58°C, and 30 s at 72°C). One μl of each of the seven amplifications was taken for the double-nested PCR with the corresponding D1N1 to D7N1 primer (29) and the REV 2B primer, with the same conditions as for the primary PCR except for the annealing temperature, 61°C. REV 1B/2B primers are located at the end of the known *BCL-1* sequence (GenBank accession no. S77049). All PCRs were amplified with a (4:1) mix of Taq and Vent polymerase. From the 36 samples that were positive for the direct breakpoint, 13 remained negative for the reciprocal PCR.

PCR Amplification of the V(D)J Idiotype. Vex 1–7 primers constitute a set of primers designed to amplify most members of the seven human V_H families. Genomic DNA (100 ng) was taken with one of the Vex 1 to Vex 7 primers and the JHex-B primer for 30 cycles (30 s at 94°C, 30 s at 60°C, and 30 s at 72°C). One μl of each of the seven amplifications was taken for the double nested secondary PCR with the corresponding VH-1 to VH-6 primer and the JHco-B primer, with the same conditions as for the primary PCR except for the annealing temperature, 63°C, and the cycles, 25 instead of 30.

Sequencing. For the direct and reciprocal breakpoints, the PCR product was directly sequenced with an IRD-800-labeled BCL-AM and REV-2M primer as described previously (29). For the V(D)J segments, the PCR product was subcloned and then sequenced with an M13 Forward (−20) primer (Invitrogen, Groningen, The Netherlands) All of the mutations detected were verified by repeating PCR and DNA sequence analyses. One nucleotide difference with the published sequence (additional C in position 492) was noted (Segal *et al.*; Ref. 31).

BclI primers (5′ to 3′) were as follows: BCL-Cn, CTACTGAAGGACTTGTGGGTTG; BCL-An/BCL-AM, CGAGGAGCATAATTGCTGCACTG; REV-1B, GGAAGTCTCACCTAGTGGAGC; REV-2B, GGAGCAGTGAACACCAGTGC; and REV-2 M, CAGTGAACACCAGTGCCCCA.

VH primers (5′ to 3′) were as follows: VH-1, CCTCAGTGAAGGTCTCCTGCAAGG; VH-2, TCCTGCGCTGGTGAAAGCCACACA; VH-3, GGTCCCTGAGACTCTCCTGTGCA; VH-4A, TCGGAGACCCTGTCCCTCACCTGCA; VH-4B, CGCTGTCTCTGGTTACTCCATCAG; VH-5, GAAAAAGCCCGGGGAGTCTCTGAA; and VH-6, CCTGTGCCATCTCCGGGGACAGTG.

Vex primers (5′ to 3′) were as follows: Vex 1, TCTGGGGCTGAGGTGAAGAA; Vex 2, ACCTTGAAGGAGTCTGGTCC; Vex 3, GTCCCTGAGACTCTCCTGT; Vex 4: CAGGACTGGTGAAGCCTTC; Vex 5, GCTGGTGCAGTCTGGAGC; Vex 6, CAGCTGCAGCAGTCAGGTC; and Vex 7, CAGGTGCAGCTGGTGCAATC.

Statistical Analysis. T-nucleotides are defined as short sequences of at least 5 nucleotides inserted in the breakpoints, with sufficient homology to the adjacent flanking sequences to exclude their concomitant presence by chance alone. The significance of identification of T-nucleotides in each sample was estimated using a binomial test, as described previously (29). The $P \leq 0.05$ was selected as the point of high statistical significance.

RESULTS

***IgH* and *BCL-1* MTC Breakpoints.** A positive PCR result for the *BCL-1*/J_H (direct) junction was obtained in 36 (39%) of 93 patients, whereas only 23 patients (25%) were positive for the D_H/*BCL-1* (reciprocal) junction. The direct and reciprocal sequences from these 23 patients were analyzed regarding their breakpoints and junction regions (Tables 1 and 2). The D_H and J_H breakpoints show features of V(D)J-mediated recombination such as precise cleavage at the coding sequence/RSS border and nucleotide deletions at the coding end. This indicates that the breaks at the *IgH* locus occur during an attempted D_H to J_H rearrangement as described previously (25, 29).

The *BCL-1* MTC breakpoints are distributed between nucleotides 411 and 561 according to GenBank sequence S77049 published by Segal *et al.* (31). Comparison of the *BCL-1* sequences in the direct and reciprocal breakpoints revealed deletions (loss of MTC sequences) of 1–29 nucleotides in 13 (57%) of the samples. No gain or loss of nucleotides was found in one sample (4%). However, nine (39%) of the breakpoints showed MTC duplications of 1–27 nucleotides

Table 1 *Sequence library of the direct (BCL-1/J_H) junctions*

Clone no.	MTC	S[a]	*BCL-1*/MTC sequence[b]	*De novo* nucleotide additions (D regions)[c]	J_H sequence[d]	J_H	S
Germ line	1		AAAATA**ACTTACTCTTTATCTGCGTGGGATGAGATTAAACTGCGTCTTCT**		TGATGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAGGT	JH3	
Germ line	2		**TCGTGGTTTGAACGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTGGCG**		ACTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4	
Germ line	3		**TGAACGAGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA**		ACAACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH5	
Germ line	4		**AGCCCCTCTCC**CGTCACATCCCCCCGACCCTGCCACAAGGGAACCTGGGG		ATTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	
1	1	−29	AAAATAACTTACT	A**CGCGAAGT**AT	TGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH5	−17
2	1	−12	AAAATAACTTACTCTTTATCTGCGTGGGATGAG	CCTAAAA(GGGCGCGACTAC)	ATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCCTCAGGT	D1-26/JH6	−20
3	1	−1	AAAATAACTTACTCTTTATCTGCGTGGGATGAGATTAAAC	ACCGAAATTTT	AACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH5	−2
4	1	0	AAAATAACTTACTCTTTATCTGCGTGGGATGAGATTAAACTG	GA	TACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−11
5	1	+3	AAAATAACTTACTCTTTATCTGCGTGGGATGAGATTAAACTGCGTC	AAGGCCTAGTTCGA	TACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−5
6	1	+10	AAAATAACTTACTCTTTATCTGCGTGGGATGAGATTAAACTGCGTC	CCACAACGTACTAC	ATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−20
7	2	+4	TC	AGAA	ACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−15
8	2	−9	TCG	ACCTCCCCAACATT	TTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4	−5
9	2	−6	TCGTGG	CTAGAGG	CTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−4
10	2	−26	TCGTGG	CACT	TACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4	−11
11	2	+16	TCGTGGTTT	CCCA	TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4	−6
12	2	−13	TCGTGGTTTG	GGA	TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4	−6
13	2	+2	TCGTGGTTTGAA	TAACGGATTCTAGGC	ATGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAGGT	JH3	−2
14	2	+5	TCGTGGTTTGAACG	GGCTTCGGA	TACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−2
15	2	−20	TCGTGGTTTGAACGCAAGAGCTCCCTGAACA	AGTT	CTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH5	−4
16	2	−5	TCGTGGTTTGAACGCAAGAGCTCCCTGAACACCTGGCGCTGCC	GGCACTTAC	GACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4	−8
17	2	−3	TCGTGGTTTGAACGCAAGAGCTCCCTGAACACCTGGCGCTGCCAT	GCTTT	CAACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH5	−1
18	2	−3	TCGTGGTTTGAACGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTG	**GACTGGGG**	TACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−8
19	3	+2	T	A**TTGGGATTG**T	CTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−4
20	3	−1	TG	TCTTTACGGGAAAATTGGTTGC	CTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGT	JH6	−4
21	3	−1	TGAA	TTTGGGCCTGGAAC	GGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4/5	−15/−18
22	3	+27	TGAACGAGGGGAAGCCCTCCTGACAGCTGGAT	A**GGGACGGGA**A	AACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH5	−2
23	4	+1	AGCCCCTCTC		GCCAGGGAACCCTGGTCACCGTCTCCTCAGGT	JH4/5	−18/−21

[a] Status of coding end or MTC end processing (0, precise; −*n*, deletion; +*n*, duplication).
[b] For an easier handling, the *BCL-1* sequence was divided into four pieces (GenBank: S77049). The MTC region is shown in bold letters.
[c] T-nucleotides are shown in bold letters. Mismatches are underlined. D_H segments found in the direct breakpoints are shown between brackets. All sequence data are available from Genbank under accession nos. AF288850 to AF288895.
[d] The J_H/D_H sequence data are from GenBank (accession no. X97051). Mutations are underlined.

Table 2 *Sequence library of the reciprocal (D_H/MTC) junctions*

Clone no.	D_H	S[a]	D_H sequence[b]	*De novo* nucleotide additions[c]	*BCL-1*/MTC sequence[d]	MTC	S
Germ line	D2-2		AGGATATTGTAGTAGTACCAGCTGCTATACC		AAAATA**ACTTACTCTTTATCTGCGTGGGATGAGATTAAACTGCGTCTTCT**	1	
	D2-15		AGGATATTGTAGTGGTGGTAGCTGCTACTCC		**TCGTGGTTTGAACGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTGGCG**	2	
	D2-21		AGCATATTGTGGTGGTGACTGCTATTCC		**TGAACGAGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA**	3	
	D3-3		GTATTACGATTTTTGGAGTGGTTATTATACC		**AGCCCCTCTCC**CGTCACATCCCCCCGACCCTGCCACAAGGGAACCTGGGG	4	
	D3-9		GTATTACGATATTTTGACTGGTTATTATAAC				
	D3-16		GTATTATGATTACGTTTGGGGGAGTTATCGTTATACC				
	D3-22		GTATTACTATGATAGTAGTGGTTATTACTAC				
	D4-4		TGACTACAGTAACTAC				
	D4-17		TGACTACGGTGACTAC				
	D5-5		GTGGATACAGCTATGGTTAC				
	D5-12		GTGGATATAGTGGCTACGATTAC				
	D6-6		GAGTATAGCAGCTCGTCC				
1	D2-21	−0	AGCATATTGTGGTGGTGACTGCTATTCC	TACCGCTGTCGGG**ACTTCGCG**GGGTTGGGC	CGTCTTCT	1	−29
2	D2-21	−22	AGCATA	CGGGATCCCAGGAC	CTTCT	1	−12
3	D4-4	−8	TGACTACA	CAAAATTCTGTCGGCACCCCCCGACCCA	GCGTCTTCT	1	−1
4	D2-2	−3	AGGATATTGTAGTAGTACCAGCTGCTAT	GGGCC	CGTCTTCT	1	0
5	D3-3	−6	TTTTGGAGTGGTTAT	CCAAATG	GTCTTCT	1	+3
6	D3-9	−8	GTATTACGATATTTTGACTGGTT	GGAGGATTTGAG	AAACTGCGTCTTCT	1	+10
7	D3-9	−3	GTATTACGATATTTTGACTGGTTATTAT	**TTCGATTTTT**	CT	1	+4
8	D3-22	−3	GTATTACTATGATAGTAGTGGTTATTAC	AGG	CGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTGGCG	2	−9
9	D5-5	−4	GTGGATACAGCTATGG	CACCTTAAGCGGT	CGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTGGCG	2	−6
10	D3-3	−10	GTATTACGATTTTTGGAGTAGG	ACGGACTGGAA	CTGGtCGCTGCCATTGGCG	2	−26
11	D3-9	−3	ATTACGATATTTTGACTGGTTATTAT	TTTTTGGGGTTTTATCCCCTCTGAGTGGGCCGACCCCGCAGCTAA	GTCTTCT	1	+16
12	D5-12	−3	GTGGATATAGTGGCTACGAT	ACTGCCTGAAAGAGCCTTTTTATGG	CCTGAACACCTGGCGCTGCCATTGGCG	2	−13
13	D3-3	−1	GTATTACGATTTTTGGAGTGGTTATTATAC	TTTCGGGTATGGTCTCTCC	AACGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTGGCG	2	+2
14	D2-2	−3	AGGATATTGTAGTAGTACCAGCTGCTAT	TTGGGTTGGCAATCC	GAACGCAAGAGCTCCCTGAACACCTGGCGCTGCCATTGGCG	2	+5
15	D3-3	−7	TTTTGGAGTGGTTA	**CAGGGGA**	GAACGAGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA	3	−20
16	D3-16	−7	GTATTATGATTACGTTTGGGGGAGTTATCG	G**GGCATGAGGGC**C	CG	2	−5
17	D3-3	−13	GTATTACGATTTTTGGAG	TGGTTATCGGGAAAGAGGGCACCCCATACAAAGACA	CG	2	−3
18	D3-9	−2	GTATTACGATATTTTGACTGGTTATTATA	GGGT	TGAACGAGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA	3	−3
19	D2-2	−3	AGGATATTGTAGTAGTACCAGCTGCTAT	G	G	2	+2
20	D4-17	0	TGACTACGGTGACTAC	CCCTGTCGTT	ACGAGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA	3	−1
21	D6-6	−6	GAGTATAGCAGC	CCCCCGCCGAG	AGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA	3	−1
22	D4-17	−6	TGACTACGGT	AACCCCCTTGA	GAGGGGAAGCCCTCCTGACAGCTGGATGGTAGGACAAAGCCTCTA	3	+27
23	D2-15	−4	GGTAGCTGCTA	GGGCC	CCCGTCACATCCCCCCGACCCTGCCACAAGGGAACCTGGGG	4	+1

[a] Status of coding end or MTC end processing (0, precise; −*n*, deletion; +*n*, duplication).
[b] The J_H/D_H sequence data are from GenBank (accession no. X97051). Mutations are underlined.
[c] T-nucleotides are shown in bold letters. Mismatches are underlined. All sequence data are available from Genbank under accession nos. AF288850 to AF288895.
[d] For an easier handling, the *BCL-1* sequence was divided into four pieces (GenBank: S77049). The MTC region is shown in bold letters. Insertions are indicated in lower case letters.

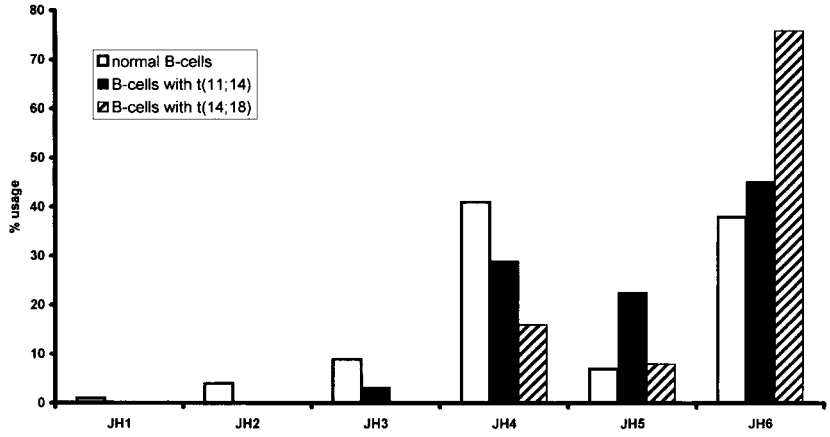

Fig. 2. Comparison of the J_H usage in the reciprocal breakpoints between normal B-cell [$n = 71$; reported by Brezinschek *et al.* (32)], MCL ($n = 31$), and FL [$n = 37$; reported by Jäger *et al.* (29)].

length. This indicates an initial staggered, double-strand break at the *BCL-1* locus that is not compatible with RSS-mediated V(D)J recombination. Thus the t(11;14) and the t(14;18) use similar mechanisms of cleavage with V(D)J-mediated breaks at the *IgH* locus, but a different pathway at the oncogene breakpoint regions.

Utilization of J_H and D_H Segments. The following J_H regions were used in the 36 direct translocation samples (Table 1; sample 24 to 36 not shown): J_H3 ($n = 1$; 3%); J_H4 ($n = 9$; 25%); J_H5 ($n = 7$; 19%); J_H6 ($n = 14$; 39%); and unclassifiable $J_H4/5$ ($n = 5$; 14%; Fig. 2). The use of J_H in the t(11;14) junctions is similar to the distribution of J_H in the idiotypes of normal B cells or B-CLL (32–34) cells with the exception of a shift from J_H4 to J_H5. Most importantly, there is no pronounced skew toward the most 3′ segment (J_H6) as in the t(14;18) in FL (70% J_H6; Ref. 29). Various D_H families were used: D_H2 (26%); D_H3 (48%); D_H4 (13%); D_H5 (9%); and D_H6 (4%). We note the low number ($n = 23$) of PCR-detectable D_H/*BCL-1* breakpoints, which may simply be attributable to the location of primers or the length of the PCR-product. On the other hand, this may reflect thus-far-unidentified structural differences at the reciprocal breakpoint. Nevertheless, the fact that 23 (64%) of 36 *BCL-1*/J_H-positive samples have a D_H/*BCL-1* reciprocal junction clearly indicates that the translocation occurs during an attempted D_H-J_H rearrangement.

T-Nucleotide Additions in *BCL-1*/*IgH* Junctions. The direct and reciprocal joining regions contained insertions of up to 45 nucleotides (Tables 1 and 2). Because we had recently noted that the t(14;18) in FL contain T-nucleotide additions, we examined the *BCL-1*/*IgH* junctions for the presence of these T-nucleotides (29). Indeed, statistically significant, nonrandom copies of the surrounding MTC, *IgH* or opposite junctional regions were found in 7 (30%) of 23 MCL samples or 7 (19%) of 37 junctions containing inserts of ≥5 *de novo* nucleotides. These T-nucleotides were 8–12 nucleotides in length and exhibited mismatches with the germ-line sequence (point mutations or deletions) in six of seven cases (Table 3; Fig. 3*A–G*). For instance, patient 1 has the 8-bp sequence ACTTCGCG in the reciprocal junction (Fig. 3*A*). The direct junction shows the same sequence in reverse complement form: CGCGAAGT. Patient 22 has the sequence GgGACGGGA in the direct junction. This is the reverse complement of the MTC sequence TCCCGTCAC with one A to G mutation (Fig. 3*G*). In contrast to the previous assumption that t(11;14) junctions contain only nontemplated N-nucleotides added by Tdt, these findings indicate the involvement of a template-dependent error-prone DNA polymerase in illegitimate recombination in at least 19% of the breakpoints. This rate is considerably less than in FL (34%; Ref. 29; Table 4).

D_H Gene Segments in the t(11;14) Junctions. As described previously, the t(11;14) junctions may contain D_H segments indicative of a preceding primary D_H-J_H rearrangement (25, 35). Applying the criteria in which sequences of 10-nucleotide lengths with a maximum of two mismatches are accepted as a D_H region (36), we could identify only two such segments [$D_H1–26$, patient 2 (Table 1); $D_H3–3$, not shown] in 36 junctions (5.6%).

Idiotypic *IgH* Sequences and T-nucleotides. The presence of T-nucleotides with their pattern of mutations and/or deletions prompted us to examine direct and reciprocal PCR-positive cases for somatic mutations in their *IgH* genes. An unequivocally monoclonal result was obtained in 11 of the 23 samples. There was a preponderance of V_H1 (4 times $V_H1–02,-08,-18$) and V_H4 (5 times $V_H4–34$ and one $V_H4–39$). One sample contained a $V_H3–21$. The rate of V_H mutations was low as expected, with an average of 1.8% (range, 0–4.8%). Only five samples had more than 2% mutations (2.4–4.8%), a rate that may be attributed to somatic hypermutation (37). T-nucleotides in the t(11;14) junctions were present in 2 of the 11 patients. Both of them had T-nucleotide mutations (patient 22) or deletions (patient 7), at V_H-mutation frequencies of 0.4 or 2.4%, respectively. On the other hand, the patient with the highest mutation frequency (patient 2; 4.8%) did not have T-nucleotides. Thus, there is no obvious association between the rate of somatic mutation and the presence of T-nucleotides at present.

DISCUSSION

The generation of a large library of direct and reciprocal t(11;14) junctions in MCLs allowed us to systematically test several hypotheses induced by a number of sporadic observations. Moreover, we are now able to put these data into the context of other B-cell lymphomas, in particular FL.

Table 3 *T-nucleotides with high significance found in the direct and reciprocal breakpoints*

Sample	Sequence[a]	Origin[b]	→[c]	Copy[d]	*P*
1	CGCGAAGT	Direct (c/r)	↔	Reciprocal	0,0289
7	TT$_a$CGAT$_a$TTTT	D_H (dir.)	→	Reciprocal	0,0396
15	C$_g$AGGGGA	MTC (dir.)	→	Reciprocal	0,0332
16	GGCAtGAGGgC	MTC (c/r)	→	Reciprocal	0,0296
18	GaCTGGGG	J_H (dir.)	→	Direct	0,0332
19	TTGGAtTG	D_H (c/r)	→	Direct	0,0178
22	GgGACGGGA	MTC (c/r)	→	Direct	0,0107

[a] Mismatches are indicated in small letters. Nucleotide deletions are specified below the line, point mutations are underlined.

[b] The original sequence is in complementary-reverse (c/r) or in direct (dir.) form.

[c] The probable templated/copy relationship is shown by a single arrow. A double arrow indicates that either sequence or the copy could be templated.

[d] Direct, nucleotide insertions in the direct junction; Reciprocal, nucleotide insertions in the reciprocal juntions.

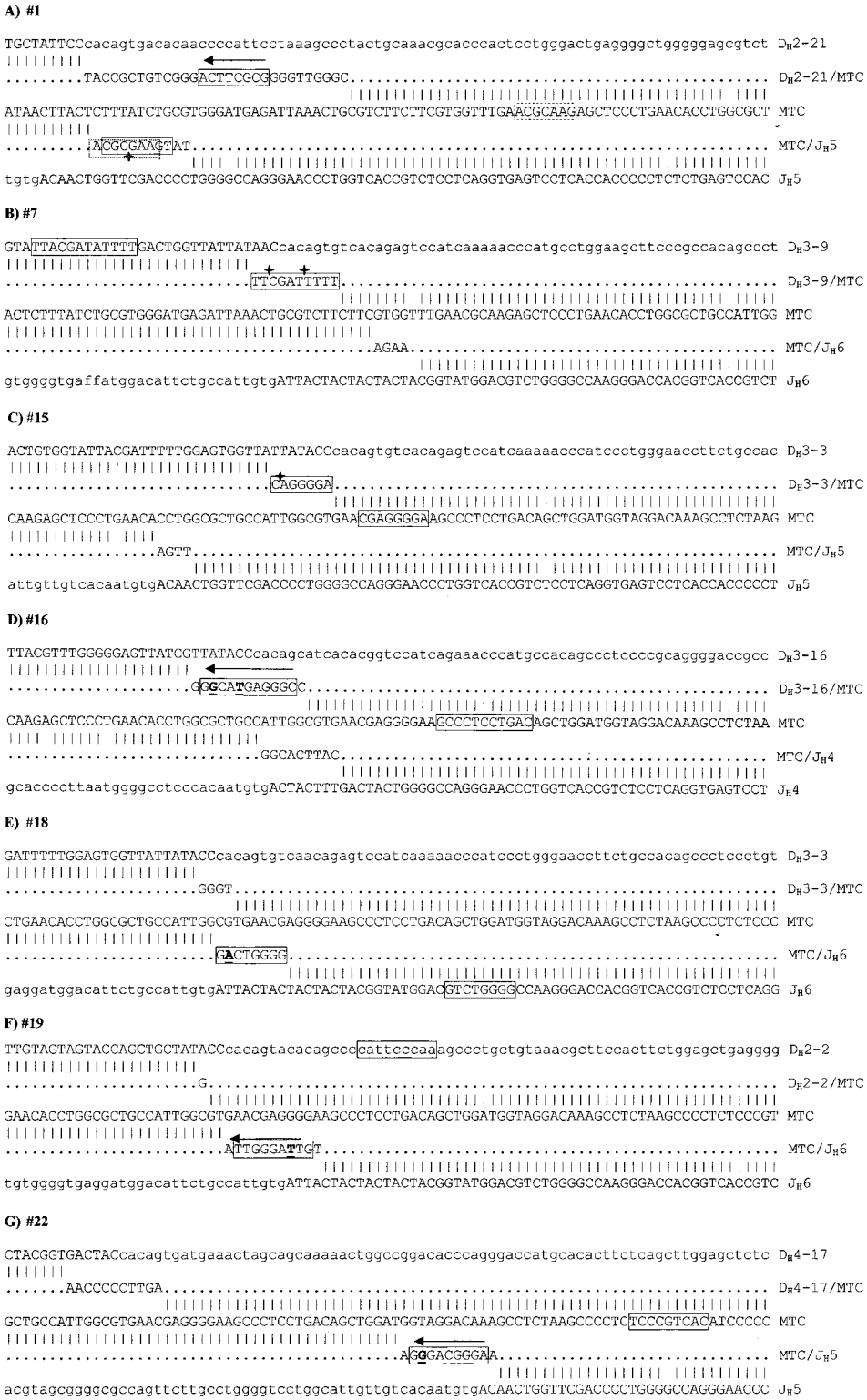

Fig. 3. *A–G*, comparison of the nucleotide sequence between the germ-line chromosome 11 (*MTC*) and 14 (D_H, J_H) and direct (*MTC*/J_H) and reciprocal (D_H/*MTC*) breakpoints resulting from the translocation of the indicated sample. *Vertical lines* and *dots*, nucleotide identity; *between dots, de novo* nucleotide insertions in the breakpoints; *lower case letters*, the remaining D_H and J_H sequences; *boxed*, homologous regions; *underlined bold letters*, mismatches; *, deletions; *arrows*, the reverse complement orientation relative to the corresponding boxed sequence.

t(11;14) Breaks Are Caused by Different Mechanisms at the *IgH* and *BCL-1* Loci. The major dogma regarding the mechanism of the t(11;14) is that V(D)J-recombination is responsible for the breaks at both the *IgH* and *BCL-1* loci (24). Some authors have postulated the presence of irregular or cryptic RSS in the *BCL-1* that could guide V(D)J-like recombination (38). The duplications of *BCL-1* sequences found in this study suggest that the breaks are attributable to an initial staggered double-strand break in at least 39% of MCLs. This is incompatible with the mechanism of V(D)J recombination, but very similar to the t(14;18), in which duplications of *BCL-2* are found in 30% of the cases (Ref. 29; Table 4). Speculation as to the mechanism of cleavage may include the transpositional activity of RAG-1/RAG-2, pathways of nonhomologous DNA end rejoining and others (39–41).

***BCL-1*/*IgH* Junctions Contain T-nucleotides.** As in the t(14;18), we also found the addition of T-nucleotides in the *BCL-1*/*IgH* junc-

Table 4 *Comparison of molecular characteristics in MCL and FL*

	MCL	FL
V(D)J mediated breaks	yes	yes[a]
BCL-breakpoint		
Duplications	9/23 (39%)	12/40 (30%)[a]
J_H usage		
J_H 4	9/36 (25%)	6/40 (15%)[a]
J_H 5	7/36 (19%)	3/40 (8%)[a]
J_H 6	14/36 (39%)	28/40 (70%)[a]
J_H unclassified	5/36 (14%)	3/40 (7%)[a]
T-nucleotides (nt)		
Samples	7/23 (30%)	17/40 (42%)[a]
Junctions with inserts ≥5nt	7/37 (19%)	23/67 (34%)[a]
$BCL/D_H/J_H$ fusion	2/36 (5,6%)	4/40 (10%)[a]
SHM (average)	1.8%	9.9%[b]

[a] Reported by Jaeger *et al.* (29).
[b] Reported by Aarts *et al.* (43).

tions. This is in contrast to previous reports on the mechanism of t(11;14), which assumed the nontemplated addition of N-nucleotides by Tdt. The presence of T-nucleotides indicates that a template-dependent, error-prone mechanism is active during illegitimate recombination, which could be involved in the repair of the ends. The recently discovered DNA polymerase μ represents an attractive canwdidate for an enzyme responsible for this phenomenon in t(11;14) as well as in t(14;18) (42). Polymerase μ is preferentially expressed in secondary lymphoid organs. Its has a template-dependent polymerase activity with a high error-rate and possesses Tdt-like activity. It is important to note that the number of T-nucleotides is considerably lower in t(11;14) than in t(14;18), as follow: 30 *versus* 42% of the samples and 19 *versus* 34% of the breakpoints with *de novo* nucleotide insertions ≥5 (Table 4). Even if it is taken into account that the T-nucleotides described here are restricted by statistical considerations, this clearly indicates differences between t(11;14) and t(14;18). These may simply be attributable to differences in the structure, location, or conformational or transcriptional status of the *BCL-1* and *BCL-2* genes at the time of translocation. Alternatively, timing of the illegitimate recombination during B-cell differentiation may be important.

Lack of Evidence for an Association between the Process of Somatic Hypermutation and the t(11;14) Mechanism. FLs have a high rate of somatic hypermutation [average, 9.9% (43)], whereas some cases of MCLs show more than 2% mutations in their *IgH* genes. However, the pattern of deletions, duplications, mutations, and T-nucleotide additions in the t(14;18) and t(11;14) junctions is strongly reminiscent of the mechanism of somatic hypermutation (44, 45). Because there may be two populations of MCLs with or without SHM, we investigated a possible association between somatic mutations in the idiotype and T-nucleotides. The patient with the highest mutation rate (patient 2) had no T-nucleotides, whereas one of two cases with T-nucleotides had a low mutation rate. Thus, there is no obvious association between the mechanism of T-nucleotide addition and the process of SHM. This is reminiscent of B-CLL, in which it has recently been shown that somatic V_H-mutations are not directly linked to the frequency of mutations in the *BCL-6* gene (46).

Stage of B-Cell Differentiation and Occurrence of t(11;14) and t(14;18). We have previously hypothesized that the t(14;18) could occur during an attempted secondary DJ_H-rearrangement in B cells that might take place in the bone marrow, but could also occur in the germinal center of the B-cell follicle (29). This is based on the observation that the t(14;18) in FL use the most 5′-D_H and the most 3′-J_H (J_H6, 71%) gene segments and has been further supported by the detection of the corresponding primary DJ_H-rearrangements in two cases of FL.[4] In contrast, the utilization of J_H regions in t(11;14) (J_H4:J_H5:J_H6, 25:19:39%) is not much different from that of normal B cells (J_H4:J_H5:J_H6, 41:7:38%; Ref. 32) or CLL cells (J_H4:J_H6, 42:37%; Ref. 34). In particular, the use of J_H6 is similar in MCL and normal B cells but is much higher in FL (J_H4:J_H5:J_H6, 15:8:70%; Ref. 29; Table 4), which suggests that the t(11;14) happens predominantly during primary rearrangements in early B cells, whereas the t(14;18) occurs mainly during an attempted secondary D_H to J_H rearrangement. This fits well with the pregerminal origin of MCL and the germinal center origin of FL. Nevertheless, the occurrence of SHM in some V(D)J junctions, the presence of rare *BCL-1/*DJ_H rearrangements, and the presence of T-nucleotides in some breakpoints might indicate that a small fraction of the t(11;14) translocations take place during an attempted secondary D_H to J_H rearrangement in a later stage of B-cell differentiation (Table 1, patient 2). This is in agreement with the previously postulated heterogeneous status of the MCL population (47).

Error-prone, Template-dependent Repair and Genetic Instability in MCL. The majority of MCL contains known aberrations that may influence genetic stability. The t(11;14) is accompanied by mutations and/or deletions of *p53*, the CDK-inhibitor family, and of the *ATM* gene (22). *ATM* deletions are found in ~40% of MCL (19, 21). Deletions or mutations of these genes involved in cell cycle regulation and DNA repair could potentially contribute to the mechanism of illegitimate recombination as shown in *ATM*-deficient patients or mice (19, 48). There is evidence that suggests that the *ATM* deletion/mutation may be the primary defect (23, 49). One could envision that an alternative (more primitive?), error-prone mechanism that allows illegitimate joining is activated in repair-deficient B cells. This would explain the addition of T-nucleotides in the junctions.

Altogether, our data indicate that the t(11;14) is generated by the same basic mechanism of translocation as the t(14;18) with V(D)J-mediated breaks in the *IgH* locus and double-strand, staggered breaks at the oncogene loci. MCL also shows error-prone template-dependent DNA synthesis during religation of the reciprocal chromosomes caused by an enzymatic machinery which is still unknown. However, there are distinct differences in the utilization of J_H regions as well as in the number of T-nucleotides in the translocation junctions which could well reflect the earlier B-cell (pregerminal center) origin of MCL. Although there may still be some dichotomy within the origin of MCL, we propose that the *BCL-1/IgH* translocation is predominantly generated during an attempted primary rearrangement, whereas the *BCL-2/IgH* translocation in FL mostly occurs during a secondary rearrangement at a later stage of B-cell differentiation.

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