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Rapid Communication

The Rapid Detection of Clonal T-cell Proliferations in Patients with Lymphoid Disorders

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A series of T-cell proliferations in peripheral blood, bone marrow, or tissue samples were analyzed for clonality. The technique used employs the polymerase chain reaction to amplify portions of the rearranged T-cell receptor β chain genes, using primers recognizing conserved sequences of the variable, diversity, and joining region segments. We examined 17 cases of T-cell lymphoma or leukemia; a clone was identified in 13 cases (76%) overall and in 7 of 8 cases (87.5%) in which both β-chain alleles were known to be rearranged, as shown by restriction enzyme analysis. No clonal rearrangements were detected in samples from 13 non–T-cell disorders, including B-cell lymphomas, reactive lymphoid proliferations, and nonlymphoid tumors. This method is useful for detecting clones in thymic and post-thymic T-cell malignancies and has the advantages of being extremely rapid (a result is obtained within hours of the biopsy procedure), requiring no radiolabeling, using only a small amount of tissue, and being applicable to formalin-fixed, paraffin-embedded tissue. (Am J Pathol 1991, 138:821–828)

It can be difficult to distinguish a reactive population of T lymphocytes from neoplastic proliferation, particularly in those patients with slowly progressive or stable disease such as large granular lymphocyte leukemia. Using restriction enzyme analysis and Southern blotting, clonal rearrangements of the immunoglobulin (Ig) and T-cell receptor (TCR) gene loci have been demonstrated in malignant lymphomas, but the methods are impractical for routine use in the histopathology laboratory.[1] To bring clonal analysis within the range of practical diagnostic methodology, we used the polymerase chain reaction (PCR) to amplify across the unique junctions created during the rearrangement of TCR-β chain genes. We previously described a similar approach to analyzing Ig heavy-chain gene rearrangements for the detection of clonal proliferations of B cells in patient samples.[2]

Although the TCR-β chain gene is arranged somewhat differently in the germline (Figure 1) than the Ig heavy chain gene, the pattern of rearrangement that occurs in the primitive cortical thymocyte is analogous.[3] It is a two-stage process under the action of the recombinase enzyme system. First one of the two diversity segments (D_1 and D_2) and one of 13 J region segments ($J_{1.1–1.6}$ and $J_{2.1–2.7}$) are selected and the intervening DNA is excised so that the chosen D segment lies 5′ or upstream of the chosen J segment. During this process, the enzyme terminal deoxynucleotidyltransferase inserts nucleotides between the D and J segments to form an N region, the length and sequence of which are random. This first stage usually occurs in both alleles before going onto the second stage described below.

The second stage initially takes place on only one allele. Again by a mechanism involving DNA excision or inversion,[3] one of the V region segments is brought to lie 5′ to the D–J segment. Terminal deoxynucleotidyltransferase again inserts a random sequence of bases between the V and D segments, here designated N′. The

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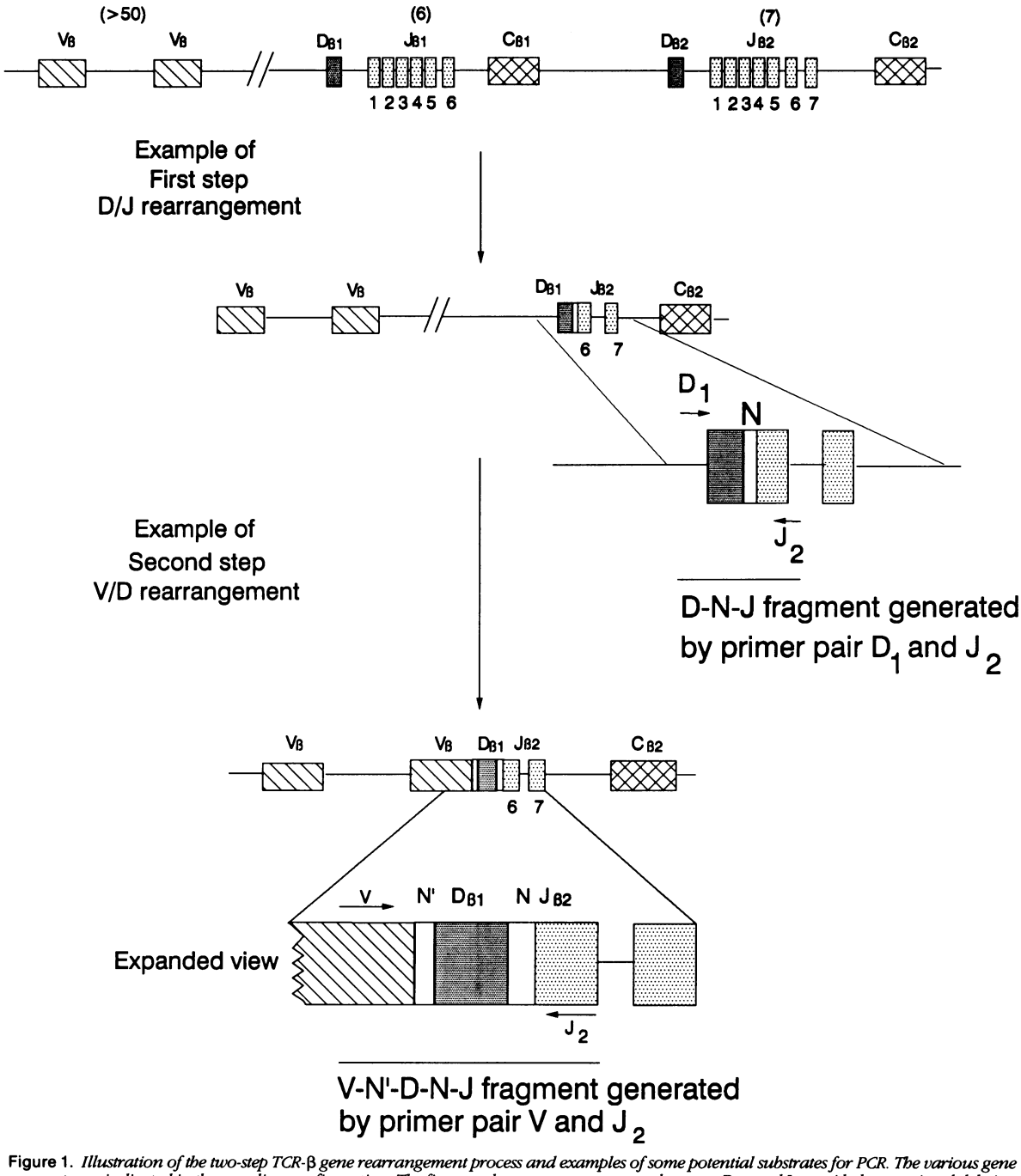

Figure 1. *Illustration of the two-step TCR-β gene rearrangement process and examples of some potential substrates for PCR. The various gene segments are indicated in the germline configuration. The first step shows a rearrangement between $D_{\beta 1}$ and $J_{\beta 2.6}$ with the associated deletion of the intervening DNA. This generates the $D_{\beta 1}$-N-$J_{\beta 2.6}$ as shown expanded on the right. If the allele remains in this configuration, the primer pair D_1/J_2 will generate a band after PCR. If the allele undergoes the second step, as indicated, a V_β gene segment is juxtaposed to $D_{\beta 1}$ and generates the V_β-N'-$D_{\beta 1}$-N-$J_{\beta 2.6}$ product, which may be amplified with the primer pair V/J_2.*

result of this two-step process is V-N'-D-N-J; this is then transcribed into RNA, processed and translated into a β-polypeptide chain that forms part of the αβ T-cell receptor. If the rearrangement does not result in the expression of a functional polypeptide, the second allele also undergoes V to D–J rearrangement.

In a manner that parallels our strategy to detect Ig heavy-chain gene rearrangements,[2] we used a primer containing a sequence common to the V segments and another complementary to a sequence present in the J segments to amplify the junction of the rearranged gene. In practice, however, no single common sequence could

be identified that was of sufficient identity to allow reliable amplification of the majority of VDJ junctions of the TCR-β–rearranged gene. Furthermore this strategy could only detect fully rearranged alleles. Therefore we adopted a more complex strategy. Two primers have been selected that bind to the J region sequences, two have been derived from the two D segment sequences, and one has been derived by the comparison of the variable segment sequences (Table 1). In this way, we hoped to detect the majority of D–J rearrangements and at least a proportion of V-D-J rearrangements.

Materials and Methods

Nine cases of leukemia and eight cases of T-cell non–Hodgkins lymphoma were studied; also included as controls were 2 cases of B-cell non–Hodgkins lymphoma, five cases of reactive lymphoid proliferation, five cases of non–lymphoid tumor, and one case of normal lymphoid tissue (Table 2). All cases had been thoroughly categorized on clinical, morphologic, and immunologic grounds. In addition, nine cases with a T-cell phenotype and three cases of polyclonal origin also had been investigated by restriction enzyme analysis for rearrangement of the TCR-β chain alleles; the DNA had been digested with various restriction enzymes, subjected to electrophoresis through an agarose gel, and transferred to nitrocellulose supported by nylon, using Southern's protocol.[4] The samples were probed using a radiolabeled 300 base-pair fragment complementary to the constant region segments of the TCR-β chain locus.[5] The details of the cases are shown in Table 2.

The leukemic samples were taken from peripheral blood, the buffy coat was separated, and then snap frozen. The remaining samples were all biopsy specimens that were snap frozen in a beaker of isopentane immersed in liquid nitrogen.

An oligomer contained within the DNA sequence at the 3′ end of published TCR-β V region sequences and found to be present in a large proportion of gene sequences reported in the literature[6–10] was chosen. Using the SEQNET facility at SERC Daresbury Laboratory, a search of a recent GenBank data base identified more than 76% of the Vβ sequences with less than four bases mismatched with the selected primer. Only members of the Vβ2, Vβ4 (and a few members of the Vβ8) gene families did not contain sufficient identity (Table 1). Also chosen were oligomeric primers contained within the published sequences of the two D region segments (including the heptamer and further sequences 5′ to the coding portions), and two primers designed to bind to the 13 J region segments[11] with varying degrees of mismatch (Table 1). Each DNA sample was analyzed with six different combinations of primers. All primer sequences are shown in Table 1, and primer pair combinations used are as listed in Table 2. D–J combinations should detect a partial or incomplete rearrangement within the TCR-β locus, while the V-J combinations should detect a proportion of completely rearranged TCR-β alleles.

DNA was extracted as previously described.[5] The PCR reaction mix was as previously described,[2] except only one unit of Taq polymerase was used in each 100-μl reaction. The PCR (30 cycles) was performed using a programmable heating block, with the following temperatures and times: 93°C for 1 minute, 55°C for 1 minute, and 73°C for 1 minute. At the end of 30 cycles, there was a 10-minute period at 55°C to allow the complete annealing of PCR products. Twenty-five-microliter aliquots then were electrophoresed through a polyacrylamide gel, stained with ethidium bromide, and viewed under ultraviolet light, as previously described.[2]

To determine sensitivity, DNA from one of the tumor samples (case 5) and from a polyclonal proliferation (case 20) or nonlymphoid tumor (case 27) were mixed in varying proportions and subjected to PCR as above.

Products of several of the PCRs were purified after agarose gel electrophoresis and sequenced directly using the protocol of Green and Giannelli[12] to confirm their authenticity. The $D\beta_1$ primer was used for sequence analyses.

Results and Discussion

We previously described a method using the PCR to detect clonal proliferations of B lymphocytes.[2] This method has proved successful in detecting a proportion of B-cell clones without fear of false-positive results, and we are now exploring strategies to increase the detection rate of true-positive results. The similarity of the rearrangement process (Figure 1) in the TCR-β chain locus makes this a logical choice to investigate using the same rationale.

In practice, however, it has proved necessary to sacrifice some of the simplicity of the original method as applied to the Ig heavy chain gene because the individual V, D, and J gene segments that compose the β-chain locus show more variability in nucleotide composition. Therefore we elected to use one V region primer, two D region primers, and two J region primers (shown in Table 1).

Positive results were taken as those cases in which there was a discrete band in at least one of the six primer pair reactions analyzed, with or without a background smear due to the presence of polyclonal, reactive lymphocytes. Bands ranged in size from 55 to 90 base pairs

Table 1. *Primers Used in this Study and Their Match to Selected V, D, and J Region Sequences*

Representatives of Vβ Families	V Primer Sequence 5′ tgtayctctgtgccagcag 3′	Match
Vβ1 (PL5.2)	TGTAT *t* TCTGTGCCAGCAG	18/19
Vβ2 (PL2.13)	TcTAC*a*TCTGc*a g t* gc *t* AG	10/19X
Vβ3 (PL4.4)	TGTACCTCTGTGCCAGCAG	19/19
Vβ4 (PL2.14)	T*a*TATCTCTGc*a g*C*g t t g a*	10/19X
Vβ5 (PL4.16)	T *t* TATCT *t* TGcGCCAGCAG	16/19
Vβ6 (PL4.14)	TGTATCTCTGTGCCAGCAG	19/19
Vβ7 (PL4.9)	TGTATCTCTGcGCCAGCAG	18/19
Vβ8 (PL3.3)	TGTAC *t* TCTGTGCCAGCAG	18/19*
Vβ9 (PL2.6)	TGTAT *t* TCTGTGCCAGCAG	18/19
Vβ10 (PL3.9)	TGTAT *t* TCTGTGCCAGCAG	18/19
Vβ11 (PL3.12)	*a*GTACCTCTGTGCCAGCA *t*	17/19
Vβ12 (PL4.2)	TGTAC *t* TCTGTGCCA*c t* AG	16/19
Vβ13 (PL4.24)	TGTAC *t* TCTGTGCCAGCAG	18/19
Vβ14 (PL8.1)	TGTAC *t* TCTGTGCCAGCAG	18/19
Vβ15 (ATL21)	T *t* TAC *t* TCTGTGCCA*c*CAG	16/19
Vβ16 (HBP42)	T *t* TAT *t* TCTGTGCCAGCAG	17/19
Vβ17 (HBVT02)	TcTATCTCTGTGCCAG *t* AG	17/19
Vβ18 (HBVT56)	*c t* TATCTCTGTGCCAGC *t c*	14/19X
Vβ19 (HBVT72)	TGTATCTCTGcGCCAGCAG	18/19
Vβ20 (HUT102)	TcTATCTCTGTGCC *t* G*g*AG	16/19

D1 Primer 5′ caaagctgtaacattgtggggac 3′

Dβ1 Sequence CTGTTTTTGTACAAAGCTGTAACATTGTG**GGGACAGGGGGC**CACAATGA

D2 Primer 5′ tcatggtgtaacattgtggggac 3′

Dβ2 Sequence ACATTTTTGTATCATGGTGTAACATTGTG**GGGACTAGCGGGAGGG**CACGATGA

J_1 Primer: 5′ acagtgagccgggttcc 3′ (t, c)

J_2 Primer: 5′ agcaccgtgagcctggtgcc 3′ (g, g)

Jβ Members	$Jβ_1$ Sense GGRACCMGGCTCACTGT	Match	$Jβ_2$ Sense GGCACCMGGCTCACGGTGCT (A/C)	Match
1.1	GG*c*ACCAG*a*CTCAC*a*GT	14/17	GGCACCAG*a*CTCACAGT *t g*T	17/20
1.2	GGGACCAGG *t* T*a*ACC*c*GT	14/17	GG*g*ACCAGG *t* T*a*ACCGT *t g*T	15/20X
1.3	GGAA*g t t* GGCTCACTGT	14/17	GG*a*A*g t t* GGCTCAC *t* GT *t g*T	13/20X
1.4	G *t* AACCC*a*GCTC *t* CTGT	14/17	G *t a*ACCC*a*GCTC *t* C *t* GT*c t*T	13/20X
1.5	GGGAC *t* CG*a*CTC *t* C*c*aT	12/17X	GGGAC *t* CG*a*CTC *t* CC*a*T*c*CT	15/20
1.6	GGGACCAGGCTCACTGT	17/17	GGGACCAGGCTCAC *t* GTG*ac*	17/20
2.1	GGGAC*a*CGGCTCAC*c*GT	15/17	GG*g*AC*a*CGGCTCACCGTGCT	18/20
2.2	GG*c t*C t AGGCT*g*AC*c*GT	12/17X	GGC *t*C *t* AGGCT*g*ACCGT*a*CT	16/20
2.3	GG*c*ACCCGGCT*g*AC*a*GT	14/17	GGCACCCGGCT*g*ACAGTGCT	19/20
2.4	GGGACCCGGCTC *t* C*a*GT	15/17	GGGACCCGGCTC *t* CAGTGCT	19/20
2.5	GG*c*ACGCGGCTC*c t g*GT	13/17	GGCACGCGGCTC*c t* AGTGCT	18/20
2.6	GG*c*A*g*CAGGCT*g*AC*c*GT	13/17	GGCA*g*CAGGCT*g*ACCGTGCT	18/20
2.7	GG*c*ACCAGGCTCAC*g*GT	15/17	GGCACCAGGCTCACGGT*c ac*	17/20

All primers are given in lower case. The opposite strands for the J primers are shown in upper case for comparison to the J region sequences. V, D, and J sequences are given in upper case, except when they do not match the relevant primer, and therefore are given in lower case, italics. M = C or A; Y = C or T; R = A or G. Matches are shown as nucleotides match/primer length; an X after the match indicates that the primer is unlikely to recognize the sequence due to percentage mismatch or poor 3′ homology. The star for primer Vβ8 family indicates that some members would not be recognized by the primer (ie, vβ8.3-8.5[17]). The bold letters in the D sequence indicate the actual D region; the underlined nucleotides are the heptamer/nonamer recognition motif. References for the representative V region sequences: Vβ1-15 in Concannon et al,[6] Vβ16 in Kimura et al,[8] Vβ17-19 in Kimura et al,[9] and Vβ20 in Kimura et al[9] and Leiden and Strominger. The J region and D region sequences are taken from Toyonaga et al.[11]

(Figure 2). This is within the range of expected band sizes (55 to 100 base pairs) as deduced from published sequences.[6–10] The V and D primers produce similar ranges of band sizing because the V primer is at the 3′ end of the V region gene segments and the D primers are at the 5′ end of the D segments.

The sensitivity results are comparable with those seen with the heavy-chain amplification technique.[2] A band is

Table 2. *Clinical Details, Restriction Enzyme Analysis Data and PCR Results*

Case	Diagnosis*	Age/Sex	REA†	Primer pairs‡						PCR§
				VJ_1	VJ_2	D_1J_1	D_1J_2	D_2J_1	D_2J_2	
1.	Lymphoblastic T-NHL	12/M	1	—	—	—	—	—	—	N
2.	Centrocytelike T-NHL (CD2+, CD4−, CD8−)	48/F	2	75	75	—	70#	—	70	VD
3.	Large cell anaplastic T-NHL (CD1a+, CD2+, CD4+, CD8−, CD30+)	15/M	ND	55	—	—	—	—	—	V
4.	T-prolymphocytic leukemia (CD2+, CD3+, CD4+, CD8−)	77/M	2	75	—	—	65	—	65	VD
5.	Large granular lymphocyte leukemia (CD2+, CD3+, CD4−, CD8+)	60/M	2	—	—	—	80#	—	80	D
6.	T-prolymphocytic leukemia (CD2+, CD3+, CD4+, CD8−)	58/M	2	—	—	—	75,# 65#	—	75	DD
7.	T-prolymphocytic leukemia (CD2+, CD3+, CD4+, CD8−)	80/F	2	—	70	—	70	—	70	VD
8.	Sezary syndrome (CD2+, CD3+, CD4+, CD8−)	74/M	2	85	—	—	—	—	80	VD
9.	T-prolymphocytic leukemia (CD2−, CD3+, CD4+, CD8−)	90/M	2	—	—	—	—	—	—	N
10.	Large granular lymphocyte leukemia (CD2+, CD3+, CD4+, CD8−)	62/M	ND	—	—	—	—	—	—	N
11.	T-prolymphocytic leukemia (CD2+, CD3+, CD4−, CD8+)	55/M	2	—	—	—	70	—	75	DD
12.	Immunoblastic T-NHL (CD2+, CD4+, CD8−)	47/F	ND	—	70	—	65	—	65	VD
13.	Lymphoblastic T-NHL (CD1a+, CD4+, CD8+)	6/M	ND	—	—	—	80	—	80	D
14.	Immunoblastic T-NHL	50/M	ND	—	—	—	75	—	75	D
15.	Lymphoblastic T-NHL (CD1a+, CD2+, CD4+, CD8+)	8/M	ND	80	—	—	—	—	—	V
16.	Large granular lymphocyte leukemia (CD2+, CD3+, CD4−, CD8−)	78/M	ND	—	—	—	85	—	86	D
17.	Mixed large and small cell T-NHL (CD2+, CD4+, CD8−)	40/M	ND	—	—	—	—	—	—	N
18.	Small lymphocytic B-NHL (CD2−, CD19+)	42/M	NR	—	—	—	—	—	—	N
19.	Diffuse small cleaved cell B-NHL (CD2−, CD19+)	59/M	NR	—	—	—	—	—	—	N
20.	Follicular hyperplasia (50%)	64/F	PC	—	—	—	—	—	—	N
21.	Follicular hyperplasia (45%)	41/M	PC	—	—	—	—	—	—	N
22.	Follicular hyperplasia and sinus histiocytosis (50%)	12/M	ND	—	—	—	—	—	—	N
23.	Paracortical hyperplasia (90%)	48/F	ND	—	—	—	—	—	—	N
24.	Dermatopathic lymphadenopathy	20/M	PC	—	—	—	—	—	—	N
25.	Granulomatous lymphadenitis (60%)	36/F	ND	—	—	—	—	—	—	N
26.	Normal lymph node (55%)	45/M	ND	—	—	—	—	—	—	N
27.	Embryonal rhabdomyosarcoma	8/M	ND	—	—	—	—	—	—	N
28.	Adenocarcinoma	42/M	ND	—	—	—	—	—	—	N
29.	Adenocarcinoma	58/F	ND	—	—	—	—	—	—	N
30.	Adenocarcinoma	57/F	ND	—	—	—	—	—	—	N

* Diagnoses have been made according to published criteria.[18–20] Percentage figures in brackets after the cases of reactive lymphoid controls refer to the approximate percentage of T cells in the sample.

† REA, restriction enzyme analysis. Figures refer to number of TCR-β chain alleles clonally rearranged; ND, not determined; PC, polyclonal; NR, not rearranged.

‡ Primer pairs: VJ_1, VJ_2, D_1J_1, D_1J_2, D_2J_1, and D_2J_2 refer to primer pairs used in individual PCR analyses. Primer sequences are shown in Table 1. Numbers given in columns refer to the size of the PCR fragment generated in base pairs. Case 2 uses $J_{2.7}$; case 5 uses $J_{2.6}$; case 6: 75 base-pair fragment uses $J_{2.3}$; 65 base-pair fragment uses $J_{2.7}$.

§ PCR refers to rearrangement status as deduced from the combined results of individual primer pairs; V, allele completely rearranged; D, allele partially rearranged; N, no band detected by PCR.

T-NHL, T-cell non–Hodgkin's Lymphoma.

B-NHL, B-cell non–Hodgkin's Lymphoma.

still visible even when tumor DNA comprises only 5% of total DNA (Figure 3). Furthermore this is against a polyclonal background; in situations in which most of the DNA has come from cells that have not rearranged their TCR-β chain genes (such as bone marrow, skin, or gut), the sensitivity is much greater (Figure 3b), detectable down to 0.05% (1 in 2000 cells).

The results, as summarized in Table 2, demonstrate

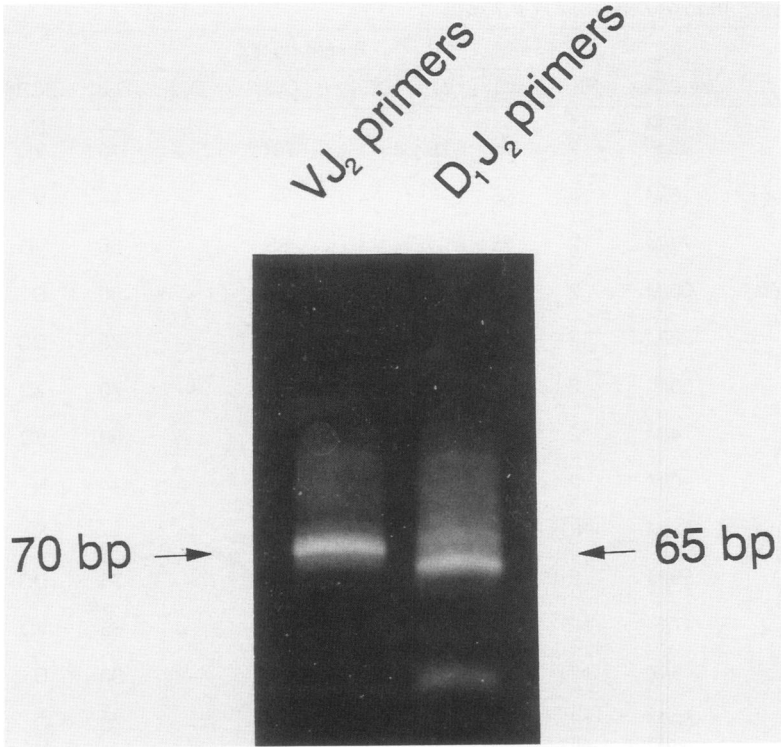

Figure 2. *Analysis of products after PCR. Ethidium bromide-stained polyacrylamide gel illustrating the analysis of case 12 in Table 2 with two different primer pairs, V/J_2 and D_1/J_2. The size of the resulting bands are shown in base pairs.*

several important features. Overall a band can be seen in one or more of the reactions in 76% of T-cell samples investigated (Figure 2 and Table 2). Where restriction enzyme analysis data are available, the system is able to detect 87.5% of T-cell tumors that have rearranged both β chain alleles; only one tumor was investigated in which it was known by restriction enzyme analysis that there was a single rearrangement and this was negative by PCR assay. The positivity rate in those cases in which restriction enzyme analysis data are not available was 75%. Of the 13 cases of non–T cell tumors, benign lymphoid proliferations or normal lymph node, no discrete bands were found. Searches of the GenBank data base with the Vβ primer revealed that the Vβ2 and Vβ4 families would probably not be recognized by this primer. A second primer (sequence 5′ TGTAYCTCTGCAGTGCTAG 3′ where Y = C,T) derived from the Vβ2 family was synthesized but failed to detect any additional positive samples in this series; therefore it was abandoned (data not shown).

In some cases the two different primer pairs produced a band of similar size. For example, in case 6, the D_1/J_2 primer pair reaction produced two bands (75 and 65 base pairs), while the D_2/J_2 primer pair reaction produced one band (75 base pairs). The sequence of the 75 base-pair band resulting from the D_1/J_2 primer pair of case 6 was determined by direct sequencing with the appropriate primers. This sequence reveals that the $D_{2.1}$ with a short N region was juxtaposed to the $J_{\beta 2.3}$ segment. Homology between the two D primers probably explains the amplification of the same allele in the two primer pair reactions. A number of other bands were sequenced to confirm their authenticity. These are identified in Table 2 with a number sign (#).

Data were available concerning CD3 membrane staining in nine cases, all of which were positive. Membrane-associated CD3 normally is found only in association with TCR, which therefore requires a complete rearrangement of the receptor gene. However cases 6 and 11 are CD3 positive, despite PCR evidence that they have only partially rearranged TCR-β alleles. This may indicate that these tumors are expressing TCR-γδ and not αβ.

No false-positive results are observed in our series; when clonal rearrangements of the TCR-β chain genes are not identified by Southern analysis, no discrete bands are present after PCR. This situation mirrors that seen when a similar technique is applied to Ig heavy-chain alleles.[2] With respect to the two B-cell tumors investigated here, it was known from restriction enzyme analysis that there were no rearrangements of the TCR-β chain loci; however it is not unusual for clonal lymphoid proliferations to rearrange antigen receptor genes inappropriate for their lineage. Thus B-cell tumors may rearrange TCR-β chain, γ chain, and δ chain genes, and T-cell tumors may rearrange immunoglobulin heavy chain

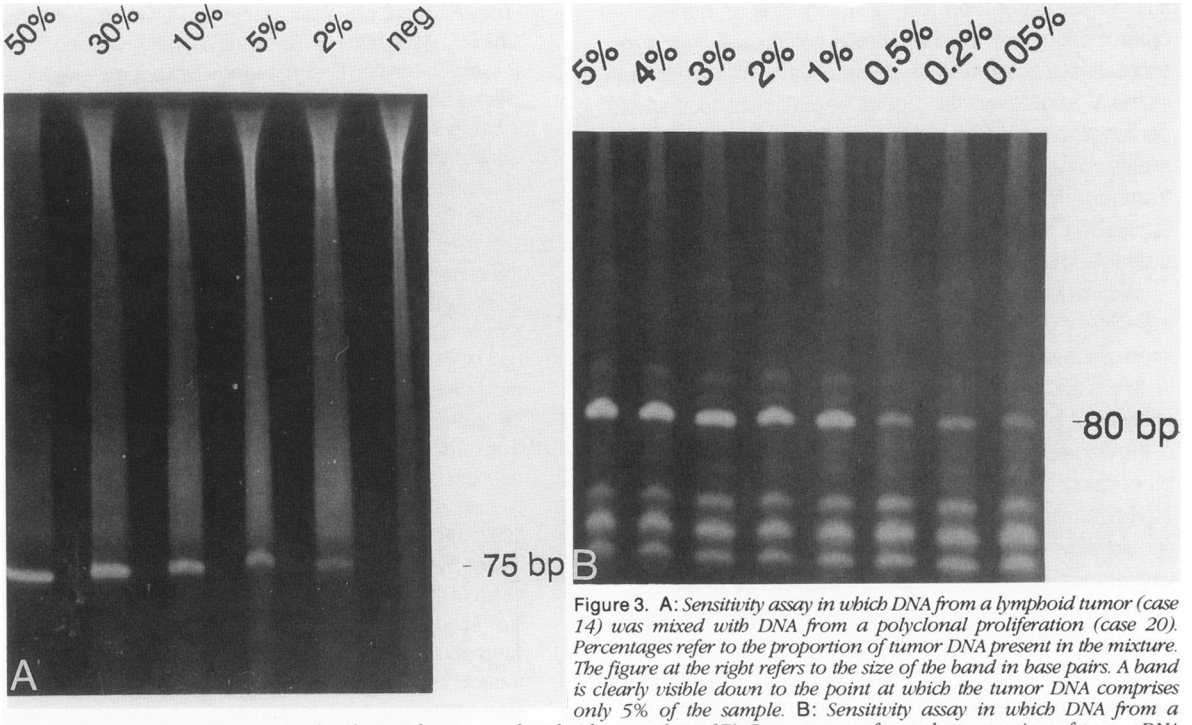

Figure 3. A: *Sensitivity assay in which DNA from a lymphoid tumor (case 14) was mixed with DNA from a polyclonal proliferation (case 20). Percentages refer to the proportion of tumor DNA present in the mixture. The figure at the right refers to the size of the band in base pairs. A band is clearly visible down to the point at which the tumor DNA comprises only 5% of the sample.* B: *Sensitivity assay in which DNA from a lymphoid tumor (case 5) was mixed with DNA from a nonlymphoid tumor (case 27). Percentages refer to the proportion of tumor DNA present in the sample. The figure at the right refers to the size of the band in base pairs. A band is still clearly visible down to the point at which the tumor DNA comprises only 0.05% of the sample.*

genes.[13,14] Such promiscuous rearrangements usually are incomplete and so it might be expected that a proportion of B-cell lesions will produce positive results by PCR, at least with the D–J primer pairs of the TCR-β loci.

A more detailed analysis of the results, with reference to individual primer pairs, reveals several important points. First the D_1/J_1 and D_2/J_1 primer pairs produced no positive results. This is surprising because the D_1/J_2 and D_2/J_2 pairs consistently did so and there is considerable sequence homology between the J primers, while the D primers differ in their sequence only at their 5′ ends. That the J_1 primer can and will bind to J segments is shown by its success in combination with the V primer. We have yet to find an explanation for this phenomenon; for example, the melting temperatures for the two primers do not differ markedly. Therefore our results show that only four of the six primer pairs used in this initial study need to be used to detect clonality in at least 75% of clonal T-cell proliferations.

The second point concerns those tumors in which both alleles are rearranged, as determined by restriction enzyme analysis. Of the eight tumors examined, five are positive with both V and D primers (numbers 2, 4, 7, 8, and 12). Because the D primers include sequences containing a portion of the recombinase recognition motif upstream of the D region, a complete or V-D-J rearrangement (which would excise the upstream sequences) would eliminate the binding site for these primers. Thus we can conclude that in these cases one allele is completely rearranged (V-D-J) and one allele is partially rearranged (D-J). Furthermore, of this group, numbers 2, 4, 7, and 12 are positive with both D_1J_2 and D_2J_2 primer pairs, indicating that these must be amplifying the same allele. This is supported by the observation that in all of the cases, save two (numbers 6 and 11), the D_1J_2 and D_2J_2 amplification products are identical in size. The explanation probably lies in the similarity between the sequences for D_1 and D_2 primers and between those for the J_1 and J_2 primers.

In the sample from case 5, despite evidence that there are two rearrangements using conventional DNA blot analysis, the only positive results with PCR are with the D_1J_2 and D_2J_2 primer pairs (each displaying a band of identical size). The most probable explanation is that there is one partial (D–J) rearrangement and one complete (V-D-J) rearrangement and that the V primer sequence does not bind to the V segment used (ie, Vβ4). Lack of V primer binding probably explains the majority of the negative cases. We intend to search for other consensus V sequences to use as primers, thus increasing the number of completely rearranged alleles that we can detect.

One explanation for lack of primer binding may be due to grossly abnormal rearrangement[15]; this could re-

sult in excision of the primer binding sites or in their incorrect orientation. Furthermore recombinase itself has exonuclease activity resulting in removal of 3′ nucleotides in the V segments; this could be sufficient to disrupt primer binding. Alternatively one of the two rearranged alleles may have been disrupted by a chromosomal translocation such as t(7;14)(q35;q32) or t(7;10)(q35;q24).[16] Unfortunately cytogenetic information is not available for most of these cases.

We investigated the utility of this technique in analyzing DNA extracted from paraffin-embedded, formalin-fixed tissue and found that the method is also applicable in this situation (data not shown). Restriction enzyme analysis of such material often is unsatisfactory because of the degree of DNA degradation that occurs during the processing of tissue; using PCR to amplify rearranged TCR-β chain and immunoglobulin heavy-chain genes should allow retrospective studies of clonal analysis in the vast archive of fixed, processed material that exists.

In conclusion, we developed a technique to amplify rearranged TCR-β chain genes and thereby detect clonal proliferations of T lymphocytes. The technique is extremely rapid, uses little DNA, does not require radiolabeled probes, and produces no false-positive results. In addition it can be applied to formalin-fixed, paraffin-embedded tissues. This technique, in conjunction with our previously published method for amplification of Ig heavy-chain genes, opens the way for the rapid clonal analysis of lymphoid proliferations to be performed in the histopathology laboratory.

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