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Application of a Multiprobe RNase Protection Assay and Junctional Sequences to Define Vβ Gene Diversity in Sezary Syndrome

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Nineteen patients with mycosis fungoides/Sezary syndrome (MF/SZ), a malignancy of the mature helper T-cell phenotype ($CD4^+TCR\alpha\beta^+$), were screened for clonotypic Vβ expansions in peripheral blood with a multiprobe RNase protection assay. A different predominant Vβ gene was identified in 9 of 14 patients with high peripheral blood CD4/CD8 ratios, whereas 4 of these patients showed T- cell expansions expressing Vβ genes other than those included in the assay. In contrast, five patients with few, if any, malignant cells in the circulation had Vβ expression levels similar to that in normal peripheral blood. A unique V-D-J sequence was found for each highly expressed Vβ gene, thereby documenting monoclonality of the expanded T-cell populations. Polymerase chain reaction (PCR) primers specific for the D-Jβ junction accurately identified the corresponding malignant clonotype in peripheral blood. The diverse TCR Vβ gene usage found in these MF/SZ patients suggests that T-cell receptor (TCR) specificity has no bearing on this disease. (Am J Pathol 1992, 140:823–830)

Human T-cell malignancies are clonal expansions of T cells arrested at distinct stages of their development.[1,2] A major means of characterizing T-cell neoplasms is analysis of their clonotypically distributed T-cell receptors (TCR, reviewed in [3,4]). These receptors are generated by the rearrangement of multiple discontinuous germline variable (V), diversity (D), and joining (J) gene segments resulting in a large number of clonotype-specific sequences. Unique rearrangements of these germline gene segments can be detected by Southern blot, thereby providing the means to identify clonal expansions of T cells and to clarify whether a malignancy is of T-cell origin.[2–5] This approach, however, has certain limitations, including: a) the inability to identify the expressed V gene, b) the inability to distinguish functional from nonfunctional rearrangements, c) the occurrence of TCR rearrangements in some cases of B-cell malignancies, and d) the relative insensitivity of the assay, which requires a minimum of 10^4 malignant cells for a detectable signal.[4]

Another approach, the use of anti-V region specific antibodies, has the advantage of determining the cell surface-expressed V gene.[6–8] This information is essential for contemplated anti-tumor V gene-specific antibody therapy. However, utilization of anti-V gene antibodies to characterize T-cell malignancies is limited by the few antibodies available[9] and their capacity to interact only with tumor cells of the mature phenotype.

Alternatively, V genes can be identified at the RNA level and we have recently developed a multiprobe RNase protection assay for 22 human Vβ genes encompassing approximately one-third of the total number of expressed human Vβ genes.[10] In this study, we applied this assay to identify the Vβ genes expressed by malignant T cells in the peripheral blood of patients with mycosis fungoides/Sezary syndrome (MF/SZ). Based on this information, we then defined the junctional sequences of the expressed Vβ genes and synthesized corresponding oligonucleotide probes. These probes proved specific and sensitive in detecting small numbers of malignant cells by the polymerase chain reaction (PCR).

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Methods

Patient Materials

Peripheral blood samples were obtained from 19 randomly selected patients (14 male, 5 female) with MF/SZ from the Yale University Dermatology Clinic (Table 1). Patients ranged in age from 39 to 82 years (mean, 62.1) with an average disease duration of 3.6 years (range, <1 to 7). Treatment was individualized according to disease severity and included photophoresis, interferon, methotrexate, and radiation. Malignant cells were immunophenotyped and all expressed the CD3 and CD4 antigens. Mononuclear cells were isolated by discontinuous gradient centrifugation using Ficoll-Hypaque. Total cellular RNA was isolated by the method of Chomczynski and Sacchi.[11] RNAs from thymocytes and peripheral blood from normal subjects were obtained as described.[10]

Oligonucleotide Primers

Oligonucleotide primers used for PCR were (5′ to 3′): Vβ2.1 CTTGGTGCTGTCGTCTCTCA, Vβ5.1 AACTTCCCTGGTCGATTCTCA, Vβ8.1 AGGTGACAGAGATGGGACAA, Vβ14.1 TCAGAATATGAACCATGAGT, Vβ18.1 TGGTACCGACAGGCTGCAGG, Vβ19.1 ACAGAATTTGAACCACGATG, Cβ AGAAGCCTGTGGCCAGGCACACCAGT, Jβ2.1 CCGAAGAACTGCTCATTG. Oligonucleotides corresponding to the junctional (D-Jβ) sequences of the β-chain genes identified in the expanded malignant T-cell population of two patients (SZ4 and SZ21) were also synthesized; $DJ\beta_{sz4}$ GCTCATTGTAGGGGGTC, and $DJ\beta_{sz21}$ CGTAGGGACCCCCCCA.

RNA Probes

Preparation of templates for generating probes of different defined lengths for the human Vβ and Cβ genes has been detailed previously.[10] The 22 Vβ probes were divided into three sets: Set A: Vβ 4.1, 5.1, 5.2, 8.1, 8.2, 11.1, 14.1, 15.1, 16.1, 17.1, 18.1, 19.1; Set B: Vβ 1.1, 3.1, 6.4, 7.1, 13.2; Set C: Vβ 2.1, 6.6, 8.3, 12.1, 13.1. Templates for each Vβ gene were separately linearized with the appropriate restriction enzyme and combined in equimolar amounts into the above probe sets. Radiolabeled RNA probes were prepared with the Riboprobe system (Promega, Madison) in 5 μl reactions using 40 ng probe set template and 75 μCi α^{32}P-UTP (15 μM final). The Cβ probe was labeled with 1/20th of the α^{32}P-UTP specific activity of the probe sets.

Multiprobe RNase Protection Assay

Assays were performed as we have previously described.[10] Briefly, 6–10 μg of total peripheral blood RNA, labeled Vβ probe set (2×10^3 cpm/uridine) and labeled Cβ probe (5×10^5 cpm) were incubated at 56°C for

Table 1. *Characteristics of MF/SZ Patients and Predominant Vβ Gene Expression in Their Peripheral Blood*

Pt.	Sex	Age	Disease duration (years)	Stage*	WBC (cells/mm^3)	CD4/8 Ratio†	Vβ‡
SZ1	F	62	6	E	12,000	63	5.1
SZ2	M	52	6	E	14,000	61	+‖
SZ3	M	64	5	E	64,000	65	2.1
SZ4	M	58	7	E	19,000	23	14.1
SZ5	F	66	5	E	11,200	28	4.1
SZ6	M	67	3	E	3,400	17	8.1
SZ7	M	81	<1	E	80,000	>99	+
SZ8	M	72	3	E+T	30,000	13	19.1
SZ12	M	76	4	E+T	8,600	4	−
SZ15	F	65	<1	E	13,000	12	+
SZ17	F	64	2	R	4,800	3	−
SZ18	M	39	3	I	6,100	4	−
SZ19	M	49	2	R	7,000	7	−
SZ21	M	43	1	I	5,500	15	18.1
SZ22	M	58	2	I	13,000	56	+
SZ23	M	82	4	E	3,200	18	−
SZ24	F	69	7	R	5,000	1	−
SZ25	M	39	3	I	8,000	13	5.2
SZ27	M	74	6	R	3,500	11	+

* E = Erythroderma; I = erythroderma improved with treatment; R = erythroderma resolved; T = tumor.
† CD4/CD8 ratio in peripheral blood.
‡ Predominant Vβ gene expansions identified in the peripheral blood. (+) = low levels of all Vβ gene genes with normal Cβ levels, (−) = normal Vβ and Cβ gene levels.
‖ Two bands in probe set B not corresponding to usual location of protected Vβ bands.

12–16 hours in 5 μl hybridization buffer (80% formamide/0.4 M NaCl/40 mM Pipes, pH 6.7). Unhyridized probe and target RNA were digested by adding 50 μl of 5 μg/ml RNase A and 10 U/ml RNase T1 in 5 mM ethylenediamine tetra acetic acid (EDTA)/0.3 M NaCl/10 mM Tris, pH 7.5, and incubating for 1 hour at 30°C. Samples were treated with 200 μg/ml proteinase K and 0.5% sodium dodecyl sulfate (SDS) at 37°C for 30 minutes, phenol-chloroform extracted, ethanol precipitated, dissolved in sample buffer, and electrophoresed on 6% polyacrylamide sequencing gels. Dried gels were exposed on Kodak XRP film at −70°C with intensifying screens for 24–72 hours. For quantitation of peak areas, autoradiograms were scanned on a laser densitometer (LKB, Piscataway, NJ).

PCR, Cloning, and Sequencing

TCR β-chain sequences were amplified with PCR using 5′ Vβ-specific and 3′ anti-sense Cβ-, Jβ- or DJβ-specific oligonucleotide primers. PCRs were performed for 30 cycles (94°C, 1 min; 55°C, 2 min; 72°C, 1 min) using the GeneAmp kit reagents and protocol (Perkin Elmer Cetus, Norwalk, CT) with each 50 μl reaction containing cDNA synthesized from 20 ng total cellular RNA and 0.4 uM primer concentrations. PCR products were purified from agarose gels, ligated into the SmaI site of pGEM-7zf (Promega, WI) and sequenced by the dideoxy method using both T7 and SP6 primers.

Calculation of Number of Clonotypic T Cells Detectable by PCR

The percent malignant cells in peripheral blood from two patients (SZ4 and SZ21) was first determined by the formula: (100%) × (A/B)[(C × D)/E], where A = Vβ peak area, B = No. uridine residues per specific Vβ probe, C = Cβ peak area, D = dilution of Cβ radiolabel (20-fold), E = No. uridine residues per Cβ probe. The minimum number of T cells necessary for a detectable PCR product was determined by the formula: Q × R × (S/100)/T, where Q = No. cells per μg RNA (≈10^6 cells/μg), R = μg RNA used to synthesize cDNA, S = % SZ cells, T = lowest dilution of cDNA resulting in a detectable PCR product.

Results

Vβ Gene Expression Profiles of Normal Peripheral Blood Samples

We have previously established the optimal conditions for our RNase protection assay in human thymocytes and have documented its linearity as well as specificity for even a single nucleotide mismatch.[10] When peripheral blood RNA samples from four normal donors were tested, a unique band developed for each of the 22 Vβ genes (Figure 1). In general, the expression levels varied widely within a given subject (e.g., in set A: Vβ 8.2, 5.1 and 18.1), whereas the overall profiles of Vβ gene expression were similar.

Vβ Gene Expression Profiles of MF/SZ Patients

Peripheral blood RNA samples from 19 MF/SZ patients were analyzed for Vβ gene expression (Table 1). In eight patients (SZ1, 3, 4, 5, 6, 8, 21, 25), a single different Vβ gene was identified as Vβ5.1, 2.1, 14.1, 4.1, 8.1, 19.1, 18.1, or 5.2 (illustrated for five patients in Figure 2: SZ1, 3, 4, 5, 25). As shown for SZ1, 4, and 25, a single band was usually observed; however, secondary bands occurred in two instances (SZ3 and SZ5). Another patient, SZ2, had two major bands in set B located at positions that did not correspond to the expected lengths for the Vβ probes within this set (Figure 2). Malignant cells from this patient probably expressed a Vβ gene partially hybridized with one of these probes. In four other patients (SZ7, 15, 22, and 27), all Vβ-specific bands were of lower intensity than those of normal controls in spite of normal Cβ transcript levels (illustrated for one such patient in Figure 2: SZ7). This profile is consistent with the expansion of malignant cells expressing either a Vβ gene not included in the assay or a polymorphic allele. All 13 of the aforementioned patients had hematogenous dissemination, as evidenced by elevated white blood cell (WBC) counts and/or high CD4/CD8 ratios (>10). One additional patient (SZ23), with an abnormally high CD4/CD8 ratio, did not show a monoclonal expansion of malignant cells, as judged by the aforementioned criteria, i.e., neither overwhelming expression of a particular Vβ gene nor a decrease in expression of all Vβ genes relative to Cβ. In this patient, the increased CD4/CD8 ratio might be caused by polyclonal $CD4^+$ T-cell expansion related to disease, treatment, or other effects. In contrast, five other patients (SZ12, 17, 18, 19, and 24) had Vβ expression levels and profiles similar to that in normal peripheral blood (exemplified in Figure 2 with SZ24). None of these five patients had elevated WBC counts, and all had CD4/CD8 ratios approaching those in normals, thereby suggesting the presence of few, if any, malignant cells in the circulation. Overall, in 14 MF/SZ patients with abnormally high CD4/CD8 ratios, at least nine different Vβ genes were represented in the tumor cell populations.

Cloning and Sequencing of the TCR Vβ Genes Identified by RNase Protection Assay

To characterize the junctional regions and confirm the neoplastic (monoclonal) origin of these highly expressed

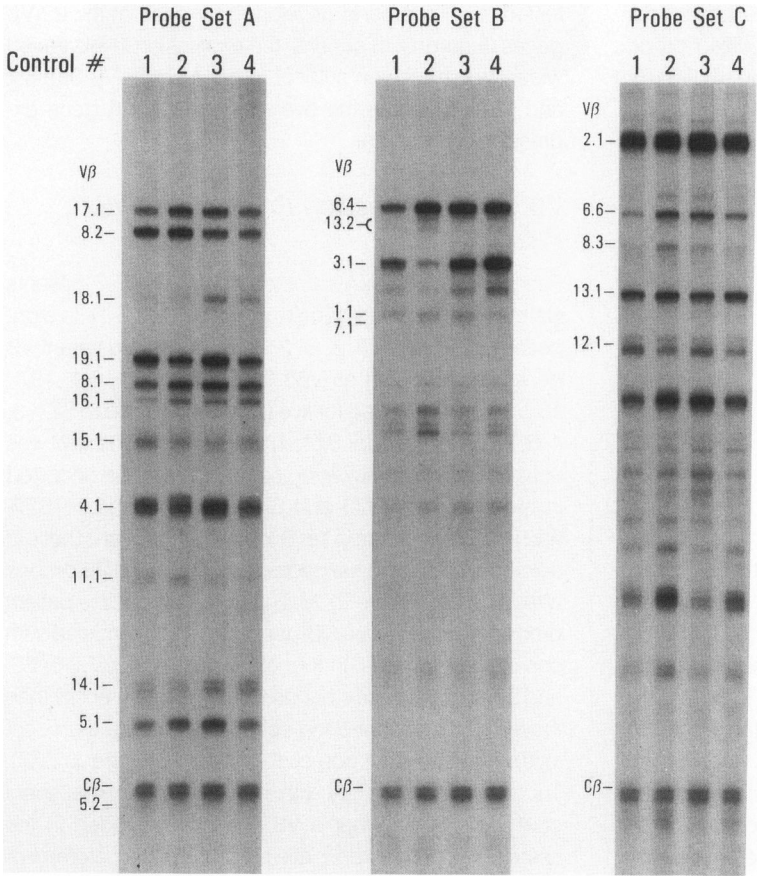

Figure 1. *Vβ multiprobe RNase protection assay of RNA from four normal donors (controls). Autoradiographs of the protected Vβ bands for the three probe sets,* **(A, B, C)** *are shown. Each lane represents the result for a single individual (#1–4). Assays were performed as described in the Methods section, on 10 μg RNA isolated from peripheral blood mononuclear cells.*

Vβ genes, β-chain genes for six patients were cloned and sequenced. Since our previous Vβ-specific TCR cloning did not yield duplicate junctional sequences, complete homology in this region for multiple clones would indicate monoclonality of the Vβ expressed in the expanded T cell population. All Vβ clones expressing the predominant Vβ in a given patient had identical junctional regions, thus verifying that these highly expressed Vβ genes resulted from monoclonal T-cell expansions (Figure 3). Furthermore, Dβ and Jβ usage appeared to be random, with both Dβ1 and Dβ2, as well as 5 of the 13 existing Jβ gene segments, utilized. All β chains had junctional sequences with open-reading frames, and based on the rule of allelic exclusion, must therefore have been the β chains expressed on the surfaces of these mature phenotype malignant T cells.

Detection of Clonotype-specific β-chains by PCR

The junctional region of the TCR is a unique clonotypic sequence that could provide a basis for developing specific probes to detect malignant T cells. Since the PCR can determine the DNA type of a single cell,[12] the feasibility of this method for detecting small numbers of tumor cells in the peripheral blood of two MF/SZ patients (SZ4 and SZ21) was evaluated. For the SZ4 β-chain, two pairs of oligonucleotide primers were used (Figure 4A), one specific for both the consensus Vβ14 and the consensus Jβ2.1 sequences, and the other for the consensus Vβ14 and the unique SZ4 junctional (D-Jβ) sequence. The Vβ14/Jβ2.1-specific primer pair could distinguish the patient with malignant cells expressing these segments from control thymocyte and peripheral blood samples of normal individuals (Figure 4B, left), yet some PCR product was detectable in these controls. Importantly, however, the Vβ14/DJβ primer pair was highly specific; only the SZ4 sample yielded a PCR product, whereas the controls did not (Figure 4B, right). Peripheral blood samples from nine additional normal individuals were also negative (not shown).

The sensitivity of the SZ4 primer pair for detecting small numbers of malignant T cells was then assessed by amplifying duplicate fivefold serial dilutions of SZ4 cDNA under the same PCR conditions (Figure 4C). In this instance, the product was detectable with as little as a

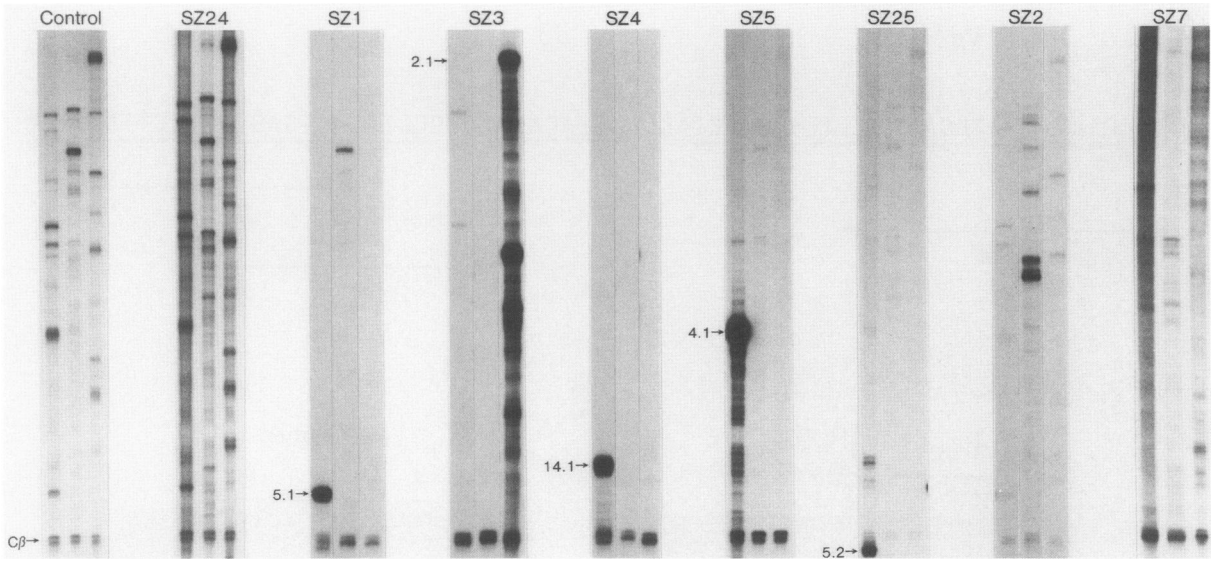

Figure 2. *Representative Vβ multiprobe RNase protection assays for seven MF/SZ patients and a normal control. Autoradiographs of protected bands are shown in groups of three probe sets (***A, B, C,** *respectively) for the individuals as indicated. The assay was performed with 7–10 μg of peripheral blood mononuclear cell RNA.*

1:625 dilution of cDNA corresponding to 32 fg of total cellular RNA. Based on this value, the percent of tumor cells in the peripheral blood of this patient and the typical yield of 1 μg of RNA from 10^6 peripheral blood cells, as few as ≈12 malignant cells can be detected. Similar degrees of specificity and sensitivity were observed when cDNA of patient SZ21 and controls were analyzed as mentioned earlier using oligonucleotide pairs corresponding to the highly expressed Vβ18 and its corresponding DJβ junctional sequence (not shown).

Discussion

We described two advances in the use of TCR Vβ genes as markers for T-cell malignancies: a) a multiprobe RNase protection assay for identifying the Vβ genes expressed by such malignant cells, and b) a junctional sequence-based PCR as a specific and sensitive means for detecting small numbers of tumor cells and thus early disease recurrence. As a model for these applications, we tested peripheral blood mononuclear cells from patients with MF/SZ and found malignant T cells to be of diverse clonal origin.

With the goal of defining in a precise manner the TCR Vβ gene involved in abnormally expanded populations of T cells, we developed the RNase "multiprobe protection assay."[10] Important features of this method include: a) the ability to screen large numbers of Vβ genes and samples simultaneously and rapidly; b) the detection of clonotypic Vβ expansions of cells regardless of TCR surface

Figure 3. *TCR β-chain junctional regions of the predominantly expressed Vβ genes for six MF/SZ syndrome patients. β-chain fragments were obtained by PCR using the appropriate Vβ- and Cβ-specific oligonucleotide primers, cloned into the SmaI site of pGEM7zf, and then sequenced. * = number of different PCR clones sequenced. + = all junctional sequences resulted in functional gene products. @ = underlined nucleotides correspond to germline Dβ sequences.*

Pt.	No.*	V(D)J+	Vβ	Dβ@	Jβ
SZ1	3	5.1(1)2.5	TGCGCCAGCAGT C A S S	TTACCGGGACAGGG L P G Q G	AGAGACCC E T Q
SZ3	6	2.1(2)2.1	TGCAGTG C S G	GGAGGGGGGGAGGGC R G G G P	CCTACAAT Y N
SZ4	5	14.1(1)2.1	TGTGCCAGCAGT C A S S	ACCCGGGACAGGACCC T R D R T P	CCTACAAT Y N
SZ6	4	8.1(2)2.3	TGTGCCAGCAGT C A S S	TTAGTTATGCGGT L V M R Y	ATACGCAG T Q
SZ8	6	19.1(1)1.5	TGTGCCAGTA C A S N	ACCTCTCCGGGACA L S G T	AATCAGCC N Q P
SZ21	3	18.1(1)2.7	TGTGCCTGG C A W	GGGGG G G	TCCCTACG P Y E

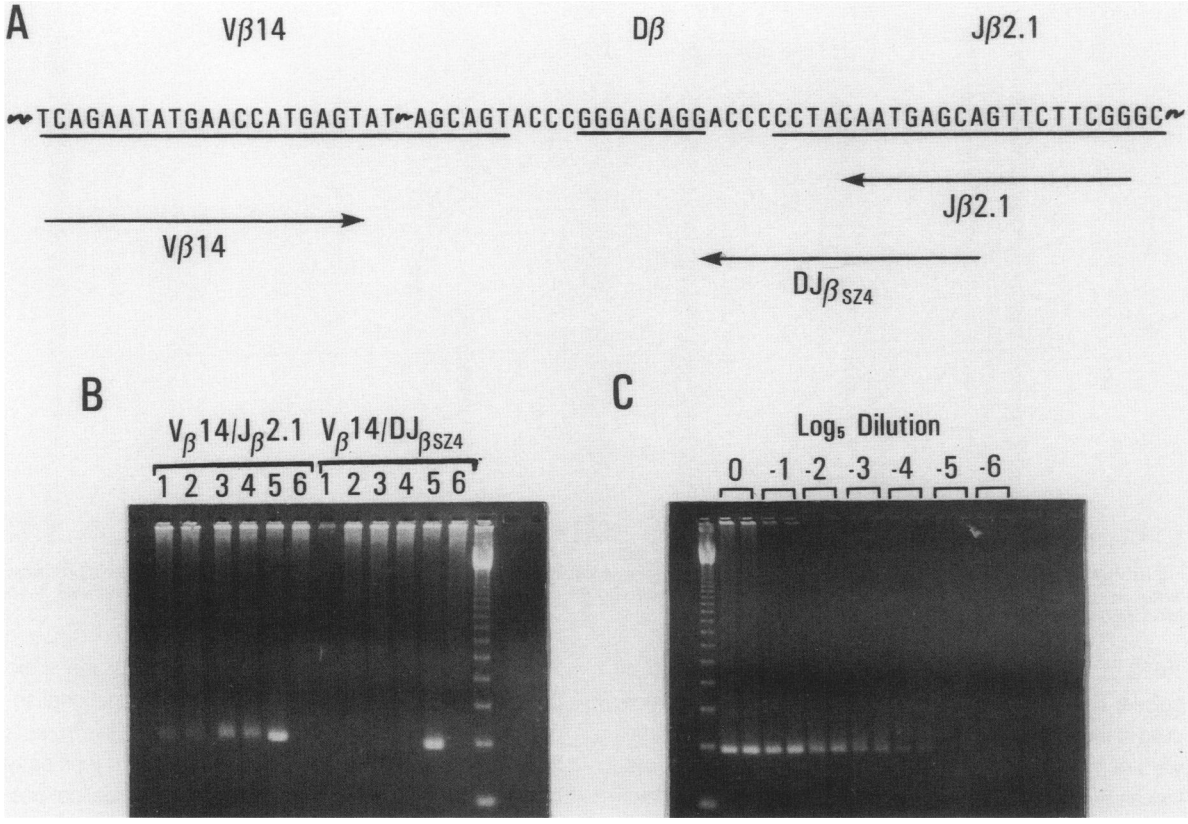

Figure 4. *PCR of TCR β-chains.* **A:** *Location of Vβ14, Jβ2.1, and DJβ$_{SZ4}$ primers on SZ4 β-chain sequence. Predicted genomic-derived nucleotide sequences for Vβ14, Dβ1, and Jβ2.1 genes are underlined. ≈ indicates locations of additional β-chain sequences not included in figure.* **B:** *PCRs were performed using Vβ14/Jβ2.1 or Vβ14/DJβ$_{SZ4}$ sets of primers to amplify cDNA from: normal peripheral blood (1, 2), thymocytes (3, 4) SZ4 peripheral blood (5), or SZ1 peripheral blood (6).* **C:** *Duplicate-fold serial dilutions of SZ4 cDNA. PCRs were performed using Vβ14 and DJβ$_{SZ4}$ primers. Dilutions are expressed as a log$_5$ value. cDNAs for PCRs were derived from the equivalent of 20 ng total cellular RNA. All PCRs were in 20 μl volumes with 10 μl of the product run on a 3% agarose gel, and then stained with ethidium bromide.*

expression, thereby extending the spectrum of characterizable malignancies to encompass immature to mature phenotypes, and c) the ability to derive quantitative results.

Four patterns of Vβ profiles were documented in the peripheral blood RNA of MF/SZ patients when analyzed by this assay: a) a profound predominance of a single Vβ gene, b) a reduced signal for all Vβs relative to the Cβ transcript levels, c) a predominant crosshybridization pattern in one of the probe sets, and d) normal Vβ gene transcript levels relative to Cβ levels. The first pattern clearly identifies the expanded clone, the second pattern strongly suggests the presence of a dominant clone whose Vβ is not, however, included in our current probe sets, and the third pattern indicates expansion of a clone expressing a Vβ highly homologous with one of our probes. The fourth pattern, of course, indicates that no significant clonal expansion has occurred.

The 22 Vβ gene probes, which represent approximately one-third the predicted Vβ repertoire,[4,13] identified a predominant Vβ gene in more than half the MF/SZ patients with sufficient numbers of atypical T cells[14] in their circulation. This may be due to a smaller than predicted Vβ gene pool size, skewing of Vβ gene usage, or the use of probes to highly expressed Vβ genes. The last possibility appears likely since 7 of the Vβ genes expressed by SZ cells are among the 9 highest expressed of the total 22 Vβ genes analyzed in normal thymocytes and peripheral blood T cells ([10] and the present study). The fact that a different Vβ gene was identified in each instance clearly indicates the absence of Vβ clonal restriction in this defined clinical entity. Although not as conclusive, others have also implied diverse Vβ usage in MF/SZ based on the heterogeneity of β-chain rearrangements observed in these patients.[15,16] These findings are in marked contrast to a recent study[8] with 2-anti-Vβ antibodies (anti-Vβ5 and anti-Vβ8), which suggested that malignant cells in skin biopsies of patients with cutaneous T-cell lymphomas utilized Vβ8 in ≈60% of the cases. This disparity may reflect the inability of antibodies to distinguish monoclonal versus polyclonal expansions or differences in specimens and clinical stage. Nevertheless, based on our findings, it appears likely that the heterogeneity of β-chain rearrangements observed in a variety

of other T-cell malignancies [2–5,17–22] is also due to diverse Vβ usage. Overall, this implies that TCR specificity has little bearing on the multiple somatic and genetic alterations currently held to result in malignant transformation.[23] Moreover, it makes it unlikely that specific Vβ molecules are the target for oncogenic infectious agents or that MF/SZ cells develop after activation by a common T-cell epitope or superantigen (antigen that activates all T cells expressing a particular Vβ gene;[24]). At this point, however, we cannot exclude the possible role of a specific pathogen with multiple T-cell antigen epitopes.

Anti-TCR antibody therapy for T-cell malignancies has been sought because of the poor overall response of T-cell neoplasms, including MF/SZ,[25–28] to current regimens. Although similar in many aspects to the anti-idiotypic therapy for B-cell malignancies,[29] anti-TCR therapy may be more effective because, unlike Ig genes of B cells, TCR Vβ genes do not utilize somatic mutations as a means of generating diversity.[30] Successful treatment of a murine model of T-cell malignancy with an anti-Vβ monoclonal antibody has been documented.[31] Of interest, such treatment resulted in longlasting resistance to further tumor challenge, possibly by permitting the hosts own anti-tumor immune response to develop. Although these studies are encouraging and emphasize the potential utility of anti-variotypic (V-region-specific) antibody therapy in this disease, our findings as to the clonal heterogeneity of MF/SZ tumor cells clearly indicates that development of a large array of antibodies to encompass most human Vβ genes will be required.

A major clinical problem in the treatment of T-cell malignancies is the identification of early disease recurrence after remission. Previous studies utilized a combination of PCR followed by hybridization of junction-specific oligonucleotide probes as a sensitive means of identifying small numbers of malignant cells in B-lineage lymphoblastic leukemia [32] or acute lymphoblastic leukemia with γδ rearrangements.[33,34] Our study further documents the validity of this approach to the follow-up of αβ TCR-expressing malignant cells. This junctional-specific PCR method, of course, requires knowledge of the β-chain sequence for each tumor. The RNase protection assay described herein, by virtue of its probe sets, specificity and sensitivity, allows easy initial identification of the tumor-associated Vβ. With this information, one can then proceed to clone the gene, characterize the junctional sequences, and produce specific oligonucleotide probes for application of the highly sensitive PCR and follow-up of tumor recurrence. Our approach should be applicable to most T-cell leukemias/lymphomas since most have rearrangements of their β-chains.[2–5] Finally, since the PCR assay can be used to detect Vβ to Jβ rearrangements at the DNA level, similar results should be obtainable with DNA from fixed tissues.

References

1. Greaves MF: Differentiation-linked leukemogenesis in lymphocytes. Science 1986, 234:697–704
2. Knowles DM: Immunophenotypic and antigen receptor gene rearrangement analysis in T cell neoplasia. Am J Pathol 1989, 134:761–785
3. Korsmeyer SJ: Antigen receptor genes as molecular marker of lymphoid neoplasms. J Clin Invest 1987, 79:1291–1295
4. Toyonaga B and TW Mak: Genes of the T-cell antigen receptor in normal and malignant cells. Ann Rev Immunol 1987, 5:585–620
5. Waldmann TA: The arrangement of immunoglobulin and T cell receptor genes in human lymphoproliferative disorders. Adv Immunol 1987, 40:247–321
6. Clark DM, Boylston AW, Hall PA, Carrel S: Antibodies to T cell antigen receptor beta chain families detect monoclonal T cell proliferation. Lancet 1986, ii:835–838
7. Salter DM, Krajewski AS, Dewar AE, Clark D, Boylston AW, Carrel S: The use of anticlonotypic T cell receptor antibodies in the diagnosis of T cell lymphoma. J Pathol 1988, 155:344A
8. Jack AS, Boylston AW, Carrel S, Grigor I: Cutaneous T-cell lymphoma cells employ a restricted range of T-cell antigen receptor variable region genes. Am J Pathol 1990, 136:17–21
9. Kappler J, Kotzin BL, Herron L, Gelfand EW, Bigler RD, Boylston A, Carrel S, Posnett DN, Choi Y, Marrack P: Vβ-specific stimulation of human T cells by staphylococcal toxins. Science 1989, 244:811–813
10. Baccala R, Kono DH, Walker S, Balderas RS, Theofilopoulos AN: Genomically imposed and somatically modified human thymocyte Vβ gene repertoires. 1991, Proc Natl Acad Sci USA 1991, 88:2908–2912
11. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987, 162:156–159
12. Li H, Gyllensten UB, Cui X, Saiki RK, Erlich HA, Arnheim N: Amplification and analysis of DNA sequences in single human sperm and diploid cells. Nature 1988, 355:414–417
13. Wilson RK, Lai E, Concannon P, Barth R, Hood LE: Structure, organization and polymorphism of murine and human T-cell receptor α and β chain gene families. Immunol Rev 1988, 101:149–172
14. Haynes BF, Metzgar RS, Minna JD, Bunn PA: Phenotypic characterization of cutaneous T-cell lymphoma. N Engl J Med 1981, 304:1319–1323
15. Ralfkiaer E, O'Connor NTJ, Crick J, Wantzin GL, Mason DY: Genotypic analysis of cutaneous T-cell lymphomas. J Invest Dermatol 1987, 88:762–765
16. Weiss LM, Wood GS, Hu E, Abel EA, Hoope RT, Sklar J: Detection of clonal T-cell gene rearrangements in the peripheral blood of patients with mycosis fungoides/Sezary syndrome. J Invest Dermatol 1989, 92:601–604
17. Flug F, Pelicci PG, Bonetti F, Knowles DM, Dalla-Favera R: T cell receptor gene rearrangements as markers of lineage and clonality in T cell neoplasms. Proc Natl Acad Sci USA 1985, 82:3460–3463

18. Waldmann TA, Davis MM, Bongiovanni KF, Korsmeyer SJ: Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. N Engl J Med 1985, 112:117–127
19. Aisenberg AC, Krontiris TS, Mak TW, Wilkes BM: Rearrangement of the gene for the beta chain of the T-cell receptor in T-cell chronic lymphocytic leukemia and related disorders. N Engl J Med 1985, 313:529–533
20. Bertness V, Kirsch I, Hollis G, Johnson B, Bunn PA: T-cell receptor gene rearrangements as clinical markers of human T-cell lymphomas. N Engl J Med 1985, 313:534–538
21. O'Connor NTJ, Weatherall DJ, Feller AC, Jones D, Pallesen G, Stein H, Wainscoat JS, Galter KC, Isaacson P, Lamert K, Ramsey A, Wright DH, Mason DY: Rearrangement of the T-cell receptor β-chain gene in the diagnosis of lymphoproliferative disorders. Lancet 1985, i:1295–1297
22. Rabbitts TH, Stinson A, Forster A, Fononi L, Luzzato L, Catovsky D, Hammarstrom L, Smith CIE, Jones D, Karpas A, Minowada J, Taylor AMR: Heterogeneity of T-cell β-chain gene rearrangements in human leukaemias and lymphomas. EMBO J 1985, 4:2217–2224
23. Nowell PC, Croce CM: Chromosome translocations and oncogenes in human lymphoid tumors. Am J Clin Pathol 1990, 94:229–237
24. Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. Science 1990, 248:705–711
25. Kaye FJ, Bunn PA, Steinberg SM, Stocker JL, Ihde DC, Fischmann AB, Glatstein EJ, Schechter GP, Phelps RM, Foss FM, Parlette HL III, Anderson MJ, Sausville EA: A randomized trial comparing combination electron-beam radiation and chemotherapy with topical therapy in the initial treatment of mycosis fungoides. N Engl J Med 1989, 321:1784–1790
26. Young RC: Mycosis fungoides. The therapeutic seach continues. N Engl J Med 1989, 321:1822–1824
27. Jones SE: Enigma of therapy for cutaneous T-cell lymphoma. J Natl Cancer Inst 1990, 82:169–170
28. Kuzel TM, Springer E, Roenigk HH, Rosen ST: Mycosis fungoides: time to define the best therapy. J Natl Cancer Inst 1990, 82:183–184
29. Levy R, Miller RA: Therapy of lymphoma directed at idiotypes. Monogr J Natl Cancer Inst 1990, 10:61–68
30. Ikuta K, Ogura T, Shimizu A, Honjo T: Low frequency of somatic mutation in β-chain variable region genes of human T-cell receptors. Proc Natl Acad Sci USA 1985, 82:7701–7704
31. Kanagawa O: *In vivo* T cell tumor therapy with monoclonal antibody directed to the Vβ chain of T cell antigen receptor. J Exp Med 1989, 170:1513–1519
32. Yamada M, Wasserman R, Lange B, Reichard BA, Womer RB, Rovera G: Minimal residual disease in childhood B-lineage lymphoblastic leukemia. Persistence of leukemic cells during the first 18 months of treatment. N Engl J Med 1990, 323:448–455
33. Hansen-Haggi TE, Hokota S, Bartram CR: Detection of minimal residual disease in acute lymphoblastic leukemia by *in vitro* amplification of rearranged T cell receptor δ chain sequences. Blood 1989, 74:1762–1767
34. Macintyre EA, d'Auriol L, Duparc N, Leverger G, Galibert F, Sigaux F: Use of oligonucleotide probes directed against T cell antigen receptor gamma delta variable-(diversity)-joining junctional sequences as a general method for detecting minimal residual disease in acute lymphoblastic leukemias. J Clin Invest 1991, 86:2125–2135