American Journal of Pathology, Vol. 149, No. 5, November 1996

Short Communication

Failure of Anti-T-Cell Receptor Vβ Antibodies to Consistently Identify a Malignant T-Cell Clone in Sézary Syndrome

Robert D. Bigler,*† Christine M. Boselli,* Brian Foley,* and Eric C. Vonderheid‡

From the Departments of Medicine, Division of Hematology/Oncology, Pathology and Laboratory Medicine,† and Dermatology,‡ Medical College of Pennsylvania and Hahnemann University, Philadelphia, Pennsylvania*

Monoclonal antibodies (MAbs) reacting with the human T cell receptor (TCR) Vβ or Vα region have been shown to be almost as specific as a private idiotypic MAb in identifying T cell clones. When available, Vβ-specific MAbs offer the ease of immunofluorescence analysis to identify and quantitate expanded malignant or nonmalignant T cell populations without requiring polymerase chain reaction (PCR) technology to evaluate expression of Vβ gene families. The Vβ expression of peripheral blood lymphocytes from twenty-three consecutive patients with Sézary syndrome has been analyzed by reverse transcriptase (RT)-PCR. Ten patients had malignant T cell clones that expressed a TCR Vβ corresponding to a commercially available anti-Vβ antibody. Immunofluorescence staining with anti-Vβ MAbs showed a direct correlation with RT-PCR results in seven of ten patients. No false positive reactivity was noted on immunofluorescence staining with any MAb. Cells from three patients, however, did not react with the corresponding anti-Vβ MAb. These three cases expressed a TCR Vβ from gene families containing a single member, ie, Vβ14, Vβ18, and Vβ20, yet MAbs reported to be specific for these regions failed to react with the T cell clone from these patients. Sequencing of the PCR product in these cases confirmed the RT-PCR results. Cells from two patients expressed a TCR using Vβ5.1-Dβ1.1 genes with different J-C segments. One patient's cells reacted with an anti-Vβ5.1 MAb (LC4) whereas the other patient's cells bound one-tenth the amount of this same MAb. These results indicate that currently available anti-TCR V region MAbs may not react consistently with T cell clones expressing the corresponding V region or may react with a low affinity making detection difficult. Differences in the J-C junction or in CDR3 may influence the binding of these MAbs. Until the false negative rate is reduced and the fine specificity and affinity of these MAbs is better characterized, both PCR and MAb studies will be required to reliably identify and quantitate clonal T cell populations. (Am J Pathol 1996, 149:1477–1483)

The T cell antigen receptor (TCR) idiotype represents a unique marker for a clone of malignant T lymphocytes. Monoclonal antibodies (MAbs) possessing private idiotypic reactivity represent the most specific antibody reagents to identify such a clone. However, production of these clonotypic reagents is time consuming and, therefore, not clinically practical. We initially demonstrated that a MAb with TCR Vβ reactivity can identify a malignant T cell in Sézary syndrome with a specificity similar to that of an anti-idiotypic MAb.[1] We further demonstrated that anti-TCR Vβ MAbs can distinguish one malignant clone from another when antibodies with appropriate specificity are available.[2] The application of anti-TCR V-region-specific antibodies to identify malignant

Supported in part by National Institutes of Health grant R01-AR40404 and The Leonard and Ruth Levine Skin Research Fund.

Accepted for publication June 17, 1996.

Address reprint requests to Dr. Robert D. Bigler, Hahnemann University, Mail Stop 412, Broad and Vine, Philadelphia, PA 19102.

clones has subsequently been confirmed in various T cell malignancies.[3–7] These reagents also have been useful in characterizing expanded T cell subpopulations expressing a limited Vβ repertoire in nonmalignant diseases.[8–11]

The lack of availability of a panel of MAbs reacting with all Vβ genes has required the use of polymerase chain reaction (PCR) technology to more fully evaluate TCR gene expression on malignant and nonmalignant T cells. Like the antibody studies, this approach also has identified monoclonal and oligoclonal T cell proliferation in various conditions.[11–15] Construction of a set of primers identifying each Vβ gene family makes this method less likely than existing MAbs to miss an expanded T cell population if present.[12,16,17] However, the ease and flexibility of immunofluorescence analysis and its ability to quantitate reactive cells compared with the requirements of PCR or reverse transcriptase (RT)-PCR suggests that antibody technology may be preferable for research and clinical use.

To compare the utility of both of these methods and evaluate the reactivity of commercially available antibodies, peripheral blood T cells from patients with Sézary syndrome were analyzed by both RT-PCR and immunofluorescence to characterize the Vβ usage by the malignant clone. This analysis of malignant T cell clones from these patients has demonstrated a limitation in the use of currently available MAbs. An unsuspected incidence of false negative results with MAb staining was noted, which raises a concern about the reliability of these reagents to consistently identify and quantitate a clonal population.

Materials and Methods

Patient Cells

Patients were diagnosed with Sézary syndrome based on clinical history and physical examination, skin biopsy, peripheral blood and/or skin immunophenotyping, and analysis of blood lymphocytes for clonal TCR gene rearrangement. All patients diagnosed with Sézary syndrome underwent leukapheresis. Lymphocytes from the leukapheresis were obtained by density gradient centrifugation (Ficoll-Paque, Pharmacia Biotech, Piscataway, NJ) and viably cryopreserved until used for analysis.

RT-PCR

An aliquot of frozen cells from the leukapheresis was rapidly thawed and total RNA extracted using RNAzol B (Cinna/Biotecx Laboratories, Houston, TX) based on the manufacturer's methodology. RT-PCR was performed as described by Choi et al[12] using the described Cβ primer in the RT reaction and the Vβ primers listed in the same study. Additional primers to detect Vβ21–24 were obtained from Dr. Mary Ann Robinson (Laboratory of Immunogenetics, National Institutes of Health). The PCR reaction was performed as described except that *Taq* and PCR buffers were obtained from GIBCO/BRL (Life Technologies, Gaithersburg, MD). The reactions were analyzed on a 3% agarose gel (Metaphor, FMC BioProducts, Rockland, ME) stained with ethidium bromide.

Immunofluorescence Analysis

Cells from the same aliquot used for RNA extraction were stained by direct or indirect immunofluorescence as described.[1] All cells were greater than 90% viable when stained. Antibodies to common T cell markers used were CD3 (Leu-4), CD4 (Leu-3a), CD2 (Leu-5b), CD8 (Leu-2a), CD7 (Leu-9), and anti-TCR-α/β-1 (WT31) (Becton Dickinson Immunocytometry Systems, San Jose, CA). The anti-TCR antibodies obtained commercially were 1C1, W112, OT145, 16G8, LC4, S511 (T Cell Diagnostics, Woburn, MA), LE-89, CAS1.1.3, BA62.6, and ELL1.4 (Immunotech, Westbrook, ME). After staining, the cells were analyzed by flow cytometry using either an Ortho Diagnostic Systems Cytofluorograf IIs or a FACScan (Becton Dickinson Immunocytometry Systems).

TCR Sequencing

Selected patients' Vβ PCR products were sequenced to evaluate the accuracy of the results obtained by PCR and MAb staining. Total RNA was amplified in a RT-PCR reaction as described above. Several reaction tubes were pooled, and the double-stranded DNA was isolated using a Wizard PCR Prep affinity column (Promega, Madison, WI). The purified product was analyzed on a 3% Metaphor gel to confirm the existence of a single band. This product was then sequenced by the DNA Sequencing Facility (University of Pennsylvania) using a nested Cβ oligonucleotide (AGCGACCTCGGGTGGGAACAC) as the sequencing primer. Sequences obtained were compared with the GenBank database, and the TCR-β genes were identified based on this analysis.

Table 1. *Correlation of RT-PCR and Anti-TCR MAb Staining of Sézary Cells*

Patient	RT-PCR, Vβ primer*	FACS reactivity†			Anti-Vβ MAb‡	
		CD3	CD4	Vβ	Vβ	Clone
EC	3	91	92	69	3	LE-89
EM	5.2–3	60	47	32	5.2–3	1C1
				1	5.3	W112
GT	6	90	91	55	6.7	OT145
MW	6	81	76	71	6.7	OT145
ER	8	86	86	79	8	16G8
MS	5.1	97	93	88	5.1	LC4
TJ	5.1	92	89	68	5.1	LC4
SS	14	95	95	0	14	CAS1.1.3
JF	18	98	98	0	18.1	BA62.6
				0	20	ELL1.4
JS	20	87	78	0	20	ELL1.4
				3	18.1	BA62.6

*Reactive Vβ primer identified by RT-PCR.
†Percentage of lymphocytes reacting with the MAb described.
‡Identification of the Vβ MAb clone reactive with the lymphocytes and the reported Vβ specificity.

Results

Cells from 23 consecutive patients with Sézary syndrome were analyzed by RT-PCR to identify the TCR Vβ gene utilized by each clone. Using the primer set available, clones have been identified in 17 patients. Failure to characterize the clones in the remaining 6 patients is due at least in part to the inability of the primer set to detect all of the currently known Vβ genes.[16] Of those patients with clones identified by RT-PCR, 10 expressed Vβ gene products for which anti-Vβ MAbs were available to evaluate for reactivity. The Vβ primer that identified the clonal population is listed (Table 1). Discrete TCR bands were not observed in reactions other than the listed Vβ primer. Results from patients with identified Vβ genes but without matching anti-Vβ MAbs are not shown.

Cells from all 10 of these patients were stained with the entire panel of antibodies listed in Materials and Methods (Table 1). The staining by anti-CD3 and anti-CD4 MAbs demonstrates that most of the T cells in the specimen express a phenotype consistent with the diagnosis of Sézary syndrome. CD7 expression did not provide a consistent marker to distinguish the malignant clone in this study as 50% of the patients had a clone that was $CD7^-$ whereas the clone was $CD7^+$ in the other patients (results not shown). Staining with Vβ-specific MAbs demonstrated that most of the $CD3^+CD4^+$ cells in 3 patients (MW, ER, and MS) were Sézary cells whereas results with 4 other patients (EC, EM, GT, and TJ) indicated that only 60% to 75% of the T cells reacted with one Vβ-specific MAb. Reactivity of the other anti-Vβ MAbs tested but not listed in the table was <5%. In these 7 cases, the specificity of the anti-Vβ MAb and the RT-PCR identification of the Vβ gene expressed by the T cell clone agreed. In contrast, cells from 3 patients failed to react with any of the anti-Vβ MAbs tested including MAbs that should have reacted based on the RT-PCR results. Due to variations in Vβ nomenclature and to illustrate the lack of cross-reactivity with other MAbs, results with both BA62.6 and ELL1.4 are reported for JF and JS, which demonstrate the absence of MAb binding in these patients. Thus, based on this selected panel of patients and MAbs, no false positive reactivity was detected nor was any cross-reactive specificity noted. However, false negative results were detected with three different MAbs.

One possible explanation for the discrepancy between the RT-PCR results and the flow cytometry studies could be the failure of the T cell clones to express the TCR. TCR expression on the surface of the cells from each patient was evaluated as part of the immunofluorescence characterization of Vβ MAb reactivity. Flow cytometric analysis of staining with CD3, CD4, the TCR-αβ MAb WT31, and the specific Vβ MAb for selected patients is illustrated in Figure 1. Analysis of patient MW is provided as a representative example of the staining observed with patients EC, EM, GT, ER, and MS. The fluorescence histograms for patient MW illustrate that a large percentage of the cells are $CD3^+CD4^+$, express the TCR, and react with the Vβ MAb detecting the Sézary clone as noted in Table 1.

In contrast to the reactivity of the Vβ MAb in patients for which the PCR and MAb studies correlate, cells from JF, JS, and SS failed to react with the anti-Vβ MAb. Expression of the TCR on cells from JF is similar to that observed in MW based on staining intensity, whereas staining of JS and SS cells is slightly less than that observed with MW. This vari-

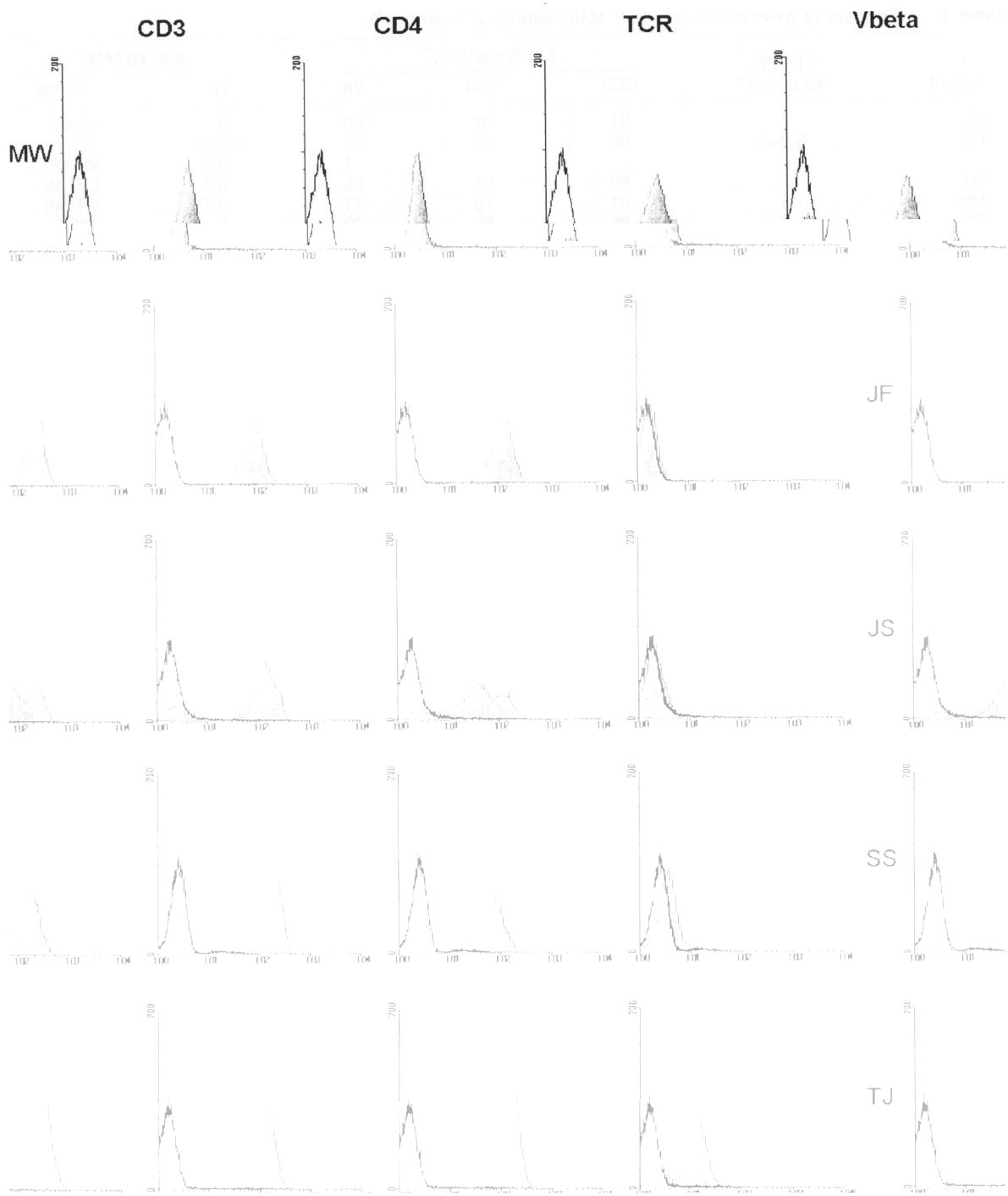

Figure 1. *Fluorescence histograms of Sézary cells analyzed for expression of T cell antigens. Cells from patients MW, JF, JS, SS, and TJ were stained with anti-CD3, anti-CD4, anti-TCR-α/β, and anti-TCR Vβ MAbs. The Vβ MAb illustrated is based on the reactive MAb for the corresponding patient listed in Table 1. Reactivity with the indicated MAb* (shaded histogram) *is compared with background fluorescence of a negative control* (open histogram). *The* x *axis is log green fluorescence.*

ability in TCR staining was observed on other cells for which Vβ MAb reactivity was demonstrated and, therefore, cannot explain the failure of Vβ-specific MAb binding in these two cases. Flow cytometric analysis of cells from patient TJ also demonstrated that almost all of the lymphocytes were $CD3^+CD4^+$ and expressed essentially normal levels of TCR-αβ. However, the staining intensity of anti-Vβ5.1(LC4) is reduced 1 log compared with the staining intensity observed with patient MW. The decrease of anti-Vβ

Table 2. *TCR-Deduced Amino Acid Sequences from Sézary Patients*

	Consensus and deduced amino acid sequence	TCR-β genes V	D	J	C
	|<——————V——————>|<—D—>|<———J———>|				
	|<—CDR 3 —>|				
Con	SETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASS SGNTIYFGEGSWLTVV	5.1		1.3	
MS	SETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASSLKVQGAGNTIYFGEGSWLTVVEDLNX	5.1	1.1	1.3	1
Con	SVSREKKERFSLILESASTNQTSMYLCASS SYEQYFGPGTRLTVT	3		2.7	
EC	XFSREEEERFSLILESASTNQTSMYLCASSSGG YYEQYFGPGTRLTXTEDLX	3	—	2.7	2
Con	ESGMPKERFSAEFPKEGPSILRIQQVVRGDSAAYFCASS NYGYTFGSGTRLTVV	18		1.2	
JF	XSGXPKGRFSSGFPQEGPSILRIQQVVRGDSAAYFCASSPKQAS NYGYTFGSGTRLTVVEDLNX	18	1.1	1.2	1
Con	ISSEVPQNLSASRPQDRQFILSSKKLLLSDSGFYLCAWS QETQYFGPGTRLLVL	20.1		2.5	
JS	VFSEVPKNLSASRPQDRQFILSSKKLLLSDSGFYLCAWGEGQG FETQDFGPGTRLLVLEDLKX	20.1*1	1.1	2.5	2
Con	SRKEKRNFPLILESPSPNQTSLYFCASS NTEAFFGQGTRLTVV	14		1.1	
SS	XFSRKEERDFPLILESPSPNQTSLYFCASSFQ GXEAFFGQGTRLTVGGTEG	14	1.1	1.1	1
Con	YFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASS SYEQYFGPGTRLTVT	5.1		2.7	
TJ	IFSETQRNXGNFPGRFSGRQFSNSRSEMNVXTLELGDSALYLCXSSLAGTG GHEQYFGPGTRLTVTEDLKX	5.1	1.1	2.7	2

Amino acid sequences were based on consensus (Con) or patient (initials) data.

MAb staining intensity suggests that LC4 may bind to TJ Sézary cells with low affinity. This low-intensity stain could result in undercounting reactive cells in a less sensitive assay and might underestimate the percentage of clonal T cells present in this case. A minimal shift in the Vβ MAb fluorescence peak compared with background fluorescence also is observed in the Vβ stain of patient SS. Although this shift is not sufficiently large to indicate definite reactivity, it could be the result of very low-affinity binding. Thus, these results confirm the expression of a TCR-αβ on the surface of all of these Sézary cells regardless of the extent of anti-Vβ MAb binding.

Due to the discrepancy between the Vβ gene expression detected by the RT-PCR and the failure of anti-Vβ MAb binding despite evidence of TCR expression on the Sézary cells, the PCR product from six of the patients was sequenced to confirm the Vβ gene identity (Table 2). Sequencing of the PCR product from two of the six patients (EC and MS) for which RT-PCR and MAb studies agreed confirmed the correlation of the RT-PCR reaction and the MAb specificity. One consistent finding in the sequences was that in the cases that had a sufficiently long D segment to be characterized the gene was identified as Dβ1.1. The J and C segments did not show this consistency and demonstrated an equal frequency of pairing Dβ1 segments with Jβ2-Cβ2 and Jβ1-Cβ1.

The sequence data for the TCR from patients SS, JF, and JS also confirmed the identification of the Vβ primer reactivity with the T cell clone in these patients. Analysis of the deduced amino acid sequences does not reveal any obvious mutation in the Vβ sequence to explain the failure of the Vβ-specific MAbs to bind to the T cell clone. The sequence obtained from the C region of SS was not of sufficient quality to unequivocally identify the Cβ gene. Therefore, assignment of the C region of the SS gene was deduced from the Jβ segment and the partial sequence obtained. Thus, the failure of the anti-Vβ14, -Vβ18, and -Vβ20 MAbs to bind to a T cell clone that expresses a TCR Vβ segment identical to that reported for the specificity of the MAb indicates that the reactivity of these MAbs does not include all clones expressing these genes.

The reduced binding of the Vβ5.1-specific MAb in TJ compared with MS also was investigated by sequencing the PCR product. The sequencing results demonstrate that both TCRs express Vβ5.1-Dβ1.1 sequences but differ in the J-C region. A comparison of the deduced amino acid sequences demonstrated relatively few differences except for expected variability in CDR3 (TJ, SLAGTGGHEQ; MS, SLKVQGAGNTI). The two receptors also used different Jβ and Cβ genes, but there was no obvious abnormalities in these genes or the J-C junction. Given the absence of any major differences in the analyzed Vβ sequence in these two cases, the decreased binding of LC4 in TJ compared with MS may be due to the differences at the J-C junction, or possibly in CDR3, altering the affinity of the MAb for the Vβ epitope.

Discussion

Anti-TCR MAbs have provided a convenient method to identify and quantitate expansion of T cell subpopulations in both malignant and nonmalignant conditions. This has been demonstrated in the T cell lymphomas and leukemias, various autoimmune diseases, infectious diseases, and others.[1–15] This study continues to support the evidence that a panel

of well characterized MAbs can permit a rapid identification of an abnormal expansion of T cells. However, the failure of several available MAbs to react with a malignant clone indicates that this lack of consistent and reliable binding must be considered in situations in which these reagents are used to identify or quantitate expanded T cell populations based on TCR Vβ expression. This is especially important when these reagents are used prospectively to identify a T cell clone in a lymphoid malignancy.

The consistency between the immunofluorescence and molecular data on 7 of 10 patients supports existing evidence on the accuracy of characterization of these MAbs and their ability to reliably detect and quantitate expanded populations of reactive T cells. However, the failure of three anti-Vβ MAbs to bind to a malignant T cell clone expressing the corresponding TCR Vβ demonstrates that absence of MAb binding currently cannot confirm the absence of a clonal population. As Vβ14, -18, and -20 are gene families containing a single member, these reagents should react with a TCR using this gene unless their reactivity is influenced by certain CDR3 sequences or an epitope dependent on J-C joining rather than an exclusive V region epitope.[16] If this is the case, then these MAbs are not specific for a TCR Vβ epitope but instead react with epitopes more narrowly defined than Vβ. This might be reflected by the manufacturer's notice about the limitation in binding of the Vβ18 MAb. An additional explanation that could support this VDJ dependency relates to the method of production of these MAbs. The CAS1.1.3 and ELL1.4 MAbs were produced using a murine T cell transfectant expressing a human Vβ gene whereas the BA62.6 MAb used a human T cell clone as the immunogen.[18,19] The transfectants were murine T cell hybridomas that expressed a TCR composed of a human Vβ gene joined to a murine Cβ gene. As the transfectant protein could express epitopes not present on the native human TCR or could express different epitopes based on the joining site in the fusion gene, MAbs derived using these T cell hybridomas as an immunogen may not consistently react with native epitopes. A more extensive evaluation of these anti-Vβ MAbs will be required to better determine whether the method of production limits their use to detect T cell clones.

In addition to the three MAbs that failed to bind to a T cell clone, one MAb demonstrated a markedly different level of binding on two different clones that expressed the same Vβ. The low-intensity staining of MAb LC4 on TJ cells expressing a similar Vβ5.1-Dβ1.1 segment to that on MS cells demonstrates the variability in MAb binding. As the Vβ sequence of these two clones did not reveal any obvious differences, this does not appear to be a likely site to explain this difference. However, the possibility of a mutation in the Vβ region that was not detected in the sequencing reaction could exist. The Vβ 5.1 primer used in the PCR does not produce a product that spans the entire V region and, therefore, a mutation present outside of the amplified region of the reported PCR sequence would not be detected. This discrepancy in reactivity could be caused by alteration in MAb binding specificity or affinity due to the differences in the CDR3 regions of these two receptors. Additional studies with T cell clones expressing well characterized DJC rearrangements with Vβ 5.1 will be required to investigate these possibilities.

This study indicates that anti-TCR Vβ or Vα MAbs will continue to be useful in identifying T cell clones. The failure of several MAbs to react with a clone for which they should have bound demonstrates that caution must be maintained in using MAbs in place of PCR to identify clonal populations. Anti-V-region MAbs can reliably detect a T cell clone in many situations; however, earlier studies were not structured to detect false negative results with antibody reactivity. A recent study did indirectly reveal some discrepancy between PCR and MAb studies although it was not mentioned.[20] Based on the data in this paper, 5 of the 16 patients reported with Sézary syndrome might show failure of MAb binding to clones with identified Vβ genes. This false negative rate of approximately 30% is similar to our results, given the numerical variations inherent in small samples. Thus, until a well characterized panel of MAbs is available with reactivity documented using a large number of defined T cell clones, both PCR and MAb staining should be used for clinical diagnostic and research studies evaluating TCR V gene expression. Furthermore, lack of binding of some of these MAbs does not necessarily prove the absence of an expansion of T cells using a specific V region. This limits the utility of these reagents to identify a clone when there is no guarantee that a clone exists. Consistently reliable identification and quantitation of T cell clones by immunofluorescence using anti-V-region MAbs will require the development of more reagents and a better understanding of the limitations of reactivity of each reagent before MAbs can replace PCR to detect a T cell clone.

Acknowledgments

We thank Dr. Mark Boyd and Dr. Mary Ann Robinson for their assistance in analysis and interpretation of the sequencing data.

References

1. Bigler RD, Fisher DE, Wand CY, Rinnooy-Kan EA, Kunkel HG: Idiotype-like molecules on cells of a human T cell leukemia. J Exp Med 1983, 158:1000–1005
2. Posnett DN, Bigler RD, Bushkin Y, Fisher DE, Wang CY, Mayer LF, Chiorazzi N, Kunkel HG. T cell antiidiotypic antibodies reveal differences between two human leukemias. J Exp Med 1984, 160:494–505
3. Janson CH, Tehrani MJ, Mellstedt H, Wigzell H: Anti-idiotypic monoclonal antibody to a T-cell chronic lymphatic leukemia. Cancer Immunol Immunother 1989, 23:225–232
4. Charley M, McCoy JP, Deng J-S, Jegasothy B: Anti-V region antibodies as "almost clonotypic" reagents for the study of cutaneous T cell lymphomas and leukemias. J Invest Dermatol 1990, 95:614–617
5. Poppema S, Hepperle B: Restricted V gene usage in T-cell lymphomas as detected by anti-T-cell receptor variable region reagents. Am J Pathol 1991, 138:1479–1484
6. Fivenson DP, Nickoloff BJ: Immunodiagnosis in cutaneous T cell lymphoma: how does gene expression of the variable region of the T cell receptor fit into the diagnostic and pathophysiological picture of T cell neoplasia. J Cutaneous Pathol 1992, 19:1–5
7. Preesman AH, Hu H-Z, Tilanus MGH, deGeus B, Schuurman H-J, Reitsma R, van Wichen DF, vanVloten WA, deWeger RA: T-cell receptor Vβ-family usage in primary cutaneous and primary nodal T-cell non-Hodgkin's lymphomas. J Invest Dermatol 1992, 99: 587–593
8. Heber-Katz E, Acha-Orbea H: The V-region disease hypothesis: evidence from autoimmune encephalomyelitis. Immunol Today 1989, 10:164–169
9. Kumar V, Kono DH, Urban JL, Hood L: The T-cell receptor repertoire and autoimmune diseases. Ann Rev Immunol 1989, 7:657–682
10. DerSimonian H, Sugita M, Glass DN, Maier AL, Weinblatt ME, Reme T, Brenner MB: Clonal Vα12.1 T cell expansions in the peripheral blood of rheumatoid arthritis patients. J Exp Med 1993, 177:1623–1631
11. Kotzin BL, Leung DYM, Kappler J, Marrack P: Superantigens and their potential role in human disease. Adv Immunol 1993, 54:99–166
12. Choi Y, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J: Interaction of *Staphylococcus aureus* toxin "superantigens" with human T cells. Proc Natl Acad Sci USA 1989, 86:8941–8945
13. Bahler DW, Berry G, Oksenberg J, Warnke RA, Levy R: Diversity of T-cell antigen receptor variable genes used by mycosis fungoides cells. Am J Pathol 1992, 140:1–8
14. Lessin SR, Rook AH, Rovera G: Molecular diagnosis of cutaneous T-cell lymphoma: polymerase chain reaction amplification of T-cell antigen receptor β-chain gene rearrangements. J Invest Dermatol 1991, 96: 299–302
15. Weiss LM, Hu E, Wood GS, Moulds C, Cleary ML, Warnke R, Sklar J: Clonal rearrangements of T-cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. N Engl J Med 1985, 313: 539–544
16. Wei S, Charmley P, Robinson MA, Concannon P: The extent of the human germline T-cell receptor Vβ gene segment repertoire. Immunogenetics 1994, 40: 27–36
17. Gorski J, Yassai M, Zhu X, Kissella B, Keever C, Flomenberg N: Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping: correlation with immune status. J Immunol 1994, 152:5109–5119
18. Romagné F, Besnardeau L, Malissen B: A versatile method to produce antibodies to human T cell receptor Vβ segments: frequency determination of human $V\beta2^+$ T cells that react with toxic-shock syndrome toxin-1. Eur J Immunol 1992, 22:2749–2752
19. Diu A, Romagné F, Genevée C, Rocher C, Bruneau J-M, David A, Praz F, Hercend T: Fine specificity of monoclonal antibodies directed at human T cell receptor variable regions: comparison with oligonucleotide-driven amplification for evaluation of Vβ expression. Eur J Immunol 1993, 23:1422–1429
20. Gorochov G, Bachelez H, Cayuela JM, Legac E, Laroche L, Dubertret L, Sigaux F: Expression of Vβ gene segments by Sézary cells. J Invest Dermatol 1995, 105:56–61