Flow Cytometric Assessment of TCR-Vβ Expression in the Evaluation of Peripheral Blood Involvement by T-Cell Lymphoproliferative Disorders

A Comparison With Conventional T-Cell Immunophenotyping and Molecular Genetic Techniques

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Abstract

Molecular genetic T-cell receptor (TCR) and flow cytometric analysis using antibodies to conventional T-cell antigens and TCR β-chain variable region families (TCR-Vβ) were performed in 65 peripheral blood specimens evaluated for potential involvement by a T-cell lymphoproliferative disorder (TCLPD). A normal or reactive conventional T-cell immunophenotype was present in 36 cases; TCR-Vβ flow cytometric and molecular TCR analyses were negative for clonality in 32 and 27 of these cases, respectively. In the remaining normal and reactive cases, one or both methods seemed to detect dominant cell populations in settings with limited T-cell diversity. We identified 29 TCLPDs; all studied cases had clonal molecular TCR results; 23 TCLPDs had clonal TCR-Vβ flow cytometric results; the remaining were suggestive of (n = 3) or negative (n = 3) for clonality. TCR-Vβ flow cytometric analysis is a powerful clinical laboratory tool that can be used to aid in the rapid diagnosis of peripheral blood involvement by T-cell malignant neoplasms.

Providing evidence of clonality is a fundamental element in establishing a diagnosis of peripheral blood involvement by chronic lymphoproliferative disorders. In B-cell neoplasms, the demonstration of restricted surface immunoglobulin light chain expression by flow cytometric immunophenotyping analysis is used commonly as proof of clonality. In contrast with B cells, antibody reagents to assess for T-cell receptor (TCR) “restriction” have not been available traditionally owing to the structural diversity of the TCR heterodimer. For this reason, molecular genetic analysis of TCR gene structure typically is used in the evaluation of potentially clonal T-cell lineage lymphoproliferative disorders.

TCR gene structure can be analyzed by polymerase chain reaction (PCR) and Southern blot techniques. Most PCR strategies use multiplexed primers directed against the TCR γ-chain gene owing to the relatively limited complexity of its genetic elements compared with the TCRα and TCRβ chains.1-4 However, TCR PCR analysis can yield banding patterns that seem clonal in cases without evidence of T-cell neoplasia.5 This phenomenon probably is due to the sensitivity of this semi-nested technique and might be particularly likely to occur in settings in which there is physiologic restriction of the T-cell repertoire, such as can occur in autoimmune disorders and with normal aging.6,7 TCR Southern blot analysis, typically performed with probes specific for the β-chain genes, does not seem to be associated with this same lack of diagnostic specificity. Southern blot analysis, however, has shortcomings—it is labor intensive, requires relatively large amounts of high-quality DNA, and can involve the use of radiolabeled reagents. Furthermore, neither the PCR nor Southern blot method readily permits the analysis of clonality in T-cell subsets unless preanalytic cell-sorting or cell-enrichment techniques are performed.
Fluorescently labeled antibody reagents to specific TCR β-chain variable region (TCR-\( \beta \)) family members recently have become commercially available for use in flow cytometric immunophenotyping analysis. Similar reagents previously have been demonstrated to provide evidence of clonality in established T-cell malignant neoplasms.\,8\,9 In the present study, we used commercially available TCR-\( \beta \) antibody reagents in clinical samples submitted for evaluation of potential involvement by T-cell malignant neoplasms. The results obtained by TCR-\( \beta \) flow cytometric analysis were compared with the results of flow cytometric immunophenotyping analysis using antibodies to conventional T cell–associated antigens and to the results of molecular genetic TCR analyses. Our results indicate that assessment of TCR-\( \beta \) expression by flow cytometric analysis provides a tool for establishing a diagnosis of T-cell malignancy in peripheral blood specimens that can be applied readily in a clinical laboratory. This method easily permits the evaluation of clonality in specific T-cell subsets and, in some instances, might eliminate the need for further molecular genetic testing.

**Materials and Methods**

**Case Selection**

We studied 65 peripheral blood specimens submitted to the Mayo Clinic hematopathology laboratory (Rochester, MN) for evaluation of potential involvement by a T-cell lineage lymphoproliferative disorder between January and November 2002. All specimens studied by TCR-\( \beta \) flow cytometric analysis were less than 48 hours old; in older specimens, high levels of nonspecific antibody binding precluded accurate interpretation of the results.

**Flow Cytometric T-Cell Immunophenotyping**

Fluorochrome-conjugated antibodies were used for multicolor flow cytometric T-cell immunophenotyping studies according to previously described methods.\,10 Initial 2-color flow cytometric analysis was performed using antibodies to CD45 (LCA) and the conventional T cell– and NK cell–associated antigens CD2, CD3, CD4, CD5, CD7, CD8, and CD16. When indicated, additional 3-color flow cytometric immunophenotyping studies were performed using antibodies to the cytotoxic T cell– and NK cell–associated antigens CD56, CD57, CD94, and CD161 and the killing inhibitory receptors (KIRs) CD158a, CD158b, and CD158e (p70). All of these antibody conjugates were from BD Biosciences (San Jose, CA) with the exception of the anti-CD94 antibody conjugate (Ancell, Bayport, MN).

**Flow Cytometric Analysis of TCR-\( \beta \) Expression**

For the assessment of TCR-\( \beta \) expression, a 4-color flow cytometric immunophenotyping strategy was used with antibodies to CD3 (peridinin chlorophyll-a protein, BD Biosciences) and CD8 (allophycocyanin, BD Biosciences) and the IOTest Beta Mark TCR-\( \beta \) Repertoire kit (Beckman Coulter, Miami, FL). This kit quantitates 24 different TCR-\( \beta \) specificities covering approximately 70% of the normal human TCR-\( \beta \) repertoire. The assay consisted of 8 tubes, each containing antibodies to CD3, CD8, and 3 distinct TCR-\( \beta \) specificities labeled with fluorescein isothiocyanate, phycoerythrin, and fluorescein isothiocyanate plus phycoerythrin, respectively. These studies also were attempted using an allophycocyanin-labeled antibody to CD4 rather than CD8. However, when the anti-CD4 antibody was used, the results were difficult to interpret owing to high levels of nonspecific TCR-\( \beta \) antibody binding in the gated CD4+ events, presumably owing to the presence of CD4+ monocytes (data not shown). For the TCR-\( \beta \) assay, the cells were stained, and the RBCs subsequently were lysed with Optilyse B (Immunootech 1400, Immunootech, Miami, FL). All analyses were performed on a FACSCalibur instrument (BD Biosciences).

Selective gating using CellQuest software (BD Biosciences) was used to analyze and quantify the number of cells positive for each TCR-\( \beta \) in the total CD3+ T cells and in the CD3+CD8+ and CD3+CD8− T-cell subsets. The values were compared with published normal ranges provided by the vendor that were verified in our laboratory in peripheral blood specimens from 10 healthy adult volunteers (data not shown). Based on correlation with previously published studies using similar reagents, clonality in this assay was defined as the expression of a single TCR-\( \beta \) either 10-fold above its normal maximum or by greater than 50% of the T cells in any analyzed population (total T cells, CD8+ T cells, or CD8− T cells).\,8\,9 Cases were considered suggestive of clonality by TCR-\( \beta \) flow cytometric immunophenotyping if, in any analyzed population, a single TCR-\( \beta \) was expressed by 40% to 49% of the cells or if greater than 70% of the cells failed to react with any of the TCR-\( \beta \) antibodies tested. Cases that failed to meet any of the aforementioned criteria were considered nonclonal by TCR-\( \beta \) flow cytometric immunophenotyping.

**Molecular Genetic TCR Analysis**

Molecular genetic analyses were performed on DNA extracted from peripheral blood or bone marrow samples, according to previously published techniques.\,2\,4 A semi-nested multiplex PCR assay was used with primers directed against the TCR γ-chain variable and joining region genes. In the Southern blot assay, extracted DNA was digested with the restriction endonuclease EcoR1, and rearrangement
bands were detected by using probes specific for the TCR β-chain genes (Jβ1 and Jβ2).

To establish the diagnostic specificity of TCR gene rearrangements, TCR PCR and Southern blot assays were performed on peripheral blood samples from 77 healthy adult volunteers. In 12 of these normal samples, bands were detected by the TCR PCR assay that met criteria for clonality. In none of these cases or any of the other normal samples did the Southern blot assay reveal evidence of clonality. In addition, review of our previous 5-year experience with TCR PCR and Southern blot testing in clinical samples indicated that the false-negative rate of TCR PCR studies in detecting Southern blot–positive T-cell clones in peripheral blood and bone marrow aspirate specimens was low (<5%; data not shown). These results are congruent with previous reports assessing the sensitivity and specificity of TCR PCR and Southern blot.

In light of these findings, the following algorithm was applied to molecular genetic TCR testing of peripheral blood specimens: Initial TCR PCR studies were performed in all cases, and the results were correlated with the results of conventional T-cell immunophenotyping analysis. In cases with a normal T-immunophenotype in which no clonal TCR PCR rearrangement bands were detected, Southern blot was not performed routinely. If there was a normal T-cell immunophenotype and yet a clonal TCR PCR rearrangement band was detected, Southern blot was performed. In this case, the positive TCR PCR results were interpreted to be of indeterminate pathologic significance if the result could not be confirmed by Southern blot. In cases with immunophenotypic evidence of T-cell malignancy, Southern blot studies were performed routinely only if results of the TCR PCR studies were negative. Results of the molecular genetic TCR studies (PCR and Southern blot) were interpreted as equivocal if a distinct band was detected in these assays in a background of multiple other bands of significant, but lesser, intensity.

This study was approved by the Mayo Clinic Institutional Review Board, and all patients consented to the use of their medical records for research.

### Table 1

<table>
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<th>Tube No./TCR-Vβ</th>
<th>Clone</th>
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<th>CD3+CD8+ T Cells</th>
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<td>4.34</td>
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FITC, fluorescein isothiocyanate; PE, phycoerythrin; TCR, T-cell receptor.

* All are murine monoclonal antibodies except BL37.2 and WUF24, which are rat monoclonal antibodies.
TCR-V\(_{\beta}\) Flow and Molecular Genetic TCR Analysis in Normal and Reactive T-Cell Immunophenotype Cases

We evaluated 65 peripheral blood specimens submitted for flow cytometric and molecular genetic analysis of potential involvement by a T-cell lymphoproliferative disorder (TCLPD). The results of conventional flow cytometric, TCR-V\(_{\beta}\) flow cytometric, and TCR PCR and Southern blot studies are summarized in Table 2. In 27 cases, the results of immunophenotyping analysis using antibodies to conventional T cell– and NK cell–associated antigens were normal. An additional 9 cases were interpreted to be most consistent with a reactive CD4 (1 case) or CD8 (8 cases) T-cell lymphocytosis based on slightly increased numbers of these T cells without immunophenotypic abnormalities. In the majority of the cases with a normal or reactive conventional T-cell immunophenotype, neither TCR-V\(_{\beta}\) flow cytometric nor TCR PCR or Southern blot studies revealed evidence of clonality Figure 2.

Four of the cases with a normal conventional T-cell immunophenotype had positive TCR PCR results; Southern blot TCR analysis was negative in all instances, and, therefore, the PCR results were interpreted to be of indeterminate pathologic significance. In 2 of these cases, the results of TCR-V\(_{\beta}\) flow were suggestive of clonality in the CD8+ T-cell subset: in one case owing to the expression of a single V\(_{\beta}\) by 40% to 49% of these cells and in the other to the absence of TCR-V\(_{\beta}\) immunoreactivity in more than 70% of the cells in this subset. The remaining 2 cases in this group had nonclonal TCR-V\(_{\beta}\) results; however, a detailed review of the data revealed that in each, a single V\(_{\beta}\) was expressed by greater than 20% of the cells in the CD8+ (1 case) or CD8− T cells (1 case), suggesting a slight predominance of a population of cells in a polyclonal T-cell background.

Equivocal molecular genetic results (based on the presence of discrete bands of moderate staining intensity in a polyclonal background banding pattern in the PCR and Southern blot assays) were obtained in 27 cases in the normal phenotype group and 1 of the reactive cases. The TCR-V\(_{\beta}\) flow cytometric analysis for these 3 cases revealed 1 nonclonal case, 1 case suggestive of clonality, and 1 case that met the criteria for clonality. In the case that was suggestive of clonality, a single V\(_{\beta}\) was expressed by 40% to 49% of the CD8+ T cells; the case with nonclonal TCR-V\(_{\beta}\) flow cytometric results also showed a slight excess (>20%) of 2 V\(_{\beta}\) in the CD8+ subset. In the case that met criteria for clonality, a single V\(_{\beta}\) (V\(_{\beta}\)16) was expressed by 22% of the CD8+ T cells. Although this is a relatively modest expansion, it was sufficient for clonality because this particular V\(_{\beta}\) is not expressed frequently by CD8+ T cells, and, therefore, this represented a 10-fold increase relative to the upper limit of normal (2.24%; see the “Materials and Methods” section for criteria).

In all of these cases with equivocal molecular results...
and in the cases with positive TCR PCR but negative Southern blot results and a normal conventional T-cell immunophenotype, the TCR-V_β flow cytometric result seemed to indicate T-cell oligoclonality or repertoire restriction, usually in the CD8+ T-cell subset (6 of 7 cases). Comparison of the TCR-V_β flow cytometric and molecular genetic results in these cases suggested that when there is relatively limited diversity in otherwise normal T-cell populations, molecular genetic techniques, particularly TCR PCR, are sensitive enough to preferentially detect predominant cell populations.

Clonal TCR PCR and Southern blot results were obtained in 2 of 9 cases in which conventional T-cell immunophenotyping analysis was consistent with a reactive lymphocytosis. In both cases, a single high-intensity band was seen in a background of multiple faint bands in the TCR PCR and Southern blot assays. One sample was obtained from a patient with a symptomatic HIV infection, and the other was from a patient with idiopathic thrombocytopenia purpura after splenectomy. Conventional T-cell immunophenotyping studies in both cases demonstrated increased numbers of CD8+ and CD57+ memory T cells. The results of the TCR-V_β flow cytometric studies were not consistent with clonality; however, in both, a single V_β was expressed by more than 20% of the CD8+ T cells. The presence of expanded populations of memory T cells and T-cell clones that can be detected by molecular genetic techniques has been described in HIV infection and autoimmune disorders. Comparison of the TCR-V_β results with the clonal molecular genetics results in the 2 cases described herein seems to illustrate the ability of molecular genetic studies to detect dominant cell populations in polyclonal or oligoclonal background T cells, in a manner similar to that seen in cases with positive TCR PCR and negative Southern blot results and a normal conventional T-cell immunophenotype and the cases with equivocal molecular genetic results.

TCR-V_β Flow Cytometric and Molecular Genetic TCR Analysis in TCLPDs

Conventional T-cell immunophenotyping and molecular genetic studies confirmed the presence of a TCLPD in 29 of 65 cases analyzed. These included Sézary

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Abnormal Phenotype</th>
<th>PCR Positive</th>
<th>SB Positive</th>
<th>PCR and SB Equivocal</th>
<th>TCR-V_β Results</th>
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</thead>
<tbody>
<tr>
<td>Normal (n = 27)</td>
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<td>4</td>
<td>0/17</td>
<td>2</td>
<td>Clonal</td>
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<td>Reactive (n = 9)</td>
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<td>2/9</td>
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<td>5/5</td>
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<td>“Suspicious”</td>
</tr>
<tr>
<td>T-LGL (n = 7)</td>
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<td>6</td>
<td>3/3</td>
<td>0</td>
<td>“Suspicious”</td>
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<tr>
<td>PTCL, NOS (n = 5)</td>
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<td>5</td>
<td>3/3</td>
<td>0</td>
<td>“Suspicious”</td>
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<tr>
<td>T-PLL (n = 4)</td>
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<td>0/0</td>
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<td>2/2</td>
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</table>

CD4+ TCLPD, CD4+ T-cell chronic lymphoproiferative disorder, not further subclassifiable; CTCL, cutaneous T-cell lymphoma; PCT, polymerase chain reaction; PTCL NOS, peripheral T-cell lymphoma, not otherwise specified; SB, Southern blot; TCR, T-cell receptor; T-LGL, T-cell large granular lymphocytic leukemia; T-PLL, T-cell prolymphocytic leukemia.

* SB performed only in selected instances (see the “Materials and Methods” section).

In 1 case, molecular testing was not performed (see the “Results” section).

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syndrome/cutaneous T-cell lymphoma (CTCL; 10 cases), T-cell granular lymphocytic leukemia (T-LGL; 7 cases), T-cell prolymphocytic leukemia (T-PLL; 4 cases), and peripheral T-cell lymphomas, not otherwise specified (PTCL, NOS; 5 cases). An additional 3 cases were involved by CD4+ chronic TCLPDs that could not be subclassified further based on the available clinical and pathologic data. In all of these cases, immunophenotyping with antibodies to conventional T cell– and NK cell–associated antigens (CD2, CD3, CD4, CD5, CD7, CD8, CD16), as well as with antibodies to...
other cytotoxic T-cell and NK-cell antigens when appropriate, revealed the presence of a discrete, immunophenotypically aberrant cell population, and molecular genetic studies confirmed TCR clonality. The molecular genetic and TCR-Vβ flow cytometric results for this group are summarized in Table 2.

The 10 CTCL cases were characterized by the presence of a CD8+ T-cell population with loss of CD7 expression. Other immunophenotypic abnormalities found in this group included loss of CD2 expression (n = 3) or distinctively dim (n = 2) or bright (n = 1) CD3 expression. In 5 cases a clearly clonal TCR PCR result in which there was a single bright band with little background banding was verified through correlation with available clinicopathologic data. In the remaining 5 cases, molecular genetic evidence of TCR clonality was confirmed by Southern blot analysis. Clonal TCR-Vβ flow cytometric patterns were detected in the CD8+ T cells in 6 cases of CTCL. In 2 cases, the TCR-Vβ result was considered suggestive of clonality because the vast majority (>90%) of the CD8– T cells failed to react with any of the TCR-Vβ antibodies tested. This pattern presumably was due to the presence of a T-cell clone expressing a TCR-Vβ not detected by the antibody panel. This lack of TCR-Vβ immunoreactivity was considered suggestive of clonality because a clonal result was inferred but not demonstrated directly. The remaining 2 CTCL cases had nonclonal TCR-Vβ flow cytometric patterns. In one of these cases, a single Vβ (Vβ13.2) was expressed by 30% of the CD8– T cells; however, this was insufficient for clonality by this assay. In the other case, the absolute lymphocyte count (970/µL [0.97 x 10⁹/L]) and the percentage of morphologically identifiable Sézary cells (1%) were low. In both of these cases the sensitivity of molecular genetic TCR studies seemed to be greater than that of the TCR-Vβ flow cytometric assay.

Seven cases of TCLPD had features diagnostic of T-LGL. All cases were characterized by the presence of a CD8+ T-cell population with diminished or lost expression of CD5. Aberrant coexpression of CD16 by the neoplastic cells was present in 5 cases; CD57 positivity was demonstrated in all 4 cases tested. Other detected abnormalities included diminished or lost expression of CD7 (n = 2) and restricted KIR antigen expression (n = 1). PCR and Southern blot analysis demonstrated clonal TCR gene rearrangements in 5 cases in which samples were submitted for analysis to the Mayo Clinic molecular genetics laboratory; an additional case was reported to have detectable clonal TCR gene rearrangements in studies performed at a separate institution. The remaining case was included for analysis because the abnormal T-cell population had immunophenotypic abnormalities, including restricted expression of the KIR antigen CD158a, that have been associated universally with the presence of clonal TCR gene rearrangements in T-LGL.

In 5 cases of T-LGL, including the case in which molecular genetic studies were not available, distinctly clonal patterns of TCR-Vβ expression were detected in the CD8+ T cells. The results of TCR-Vβ flow cytometric analysis for 1 case of T-LGL were interpreted as suggestive of clonality based on the failure of greater than 80% of the CD8+ T cells to react with any of the TCR-Vβ antibodies tested. In 1 case, TCR-Vβ flow cytometric analysis failed to demonstrate clonality despite the presence of demonstrable clonal TCR rearrangements by Southern blot analysis. This case was a patient with treated T-LGL in whom the absolute number of abnormal lymphocytes in the peripheral blood was very low (280/µL [0.28 x 10⁹/L]). Review of the TCR-Vβ flow cytometric data for this case revealed that greater than 20% of the CD8+ cells expressed Vβ2, a finding insufficient for clonality owing to the relatively frequent expression of this Vβ type in normal CD8+ T cells (upper limit of normal, 12.42%).

We evaluated 5 peripheral blood specimens involved by PTCL. On review of histologic material, 4 of these cases were subclassified as PTCL, unspecified type according to World Health Organization criteria. In these cases, the neoplastic T cells in the peripheral blood were CD4+. 2 cases showed loss of CD7. The remaining case of PTCL was an extranodal cytotoxic T-cell lymphoma with primary skin involvement. The neoplastic cells in this case coexpressed CD8 and CD16. The remainder of the TCLPD group included 4 cases of T-PLL, 3 of which showed dual CD4 and CD8 expression, and 3 other CD4+ TCLPDs that could not be subclassified further based on available clinical and pathologic data. In all of these PTCL cases and the other TCLPD cases, TCR-Vβ flow cytometric analysis provided unequivocal evidence of clonality in the appropriate CD8– or CD8+ T-cell subset, and these findings were confirmed by molecular genetic TCR analysis.

Discussion

In the clinical evaluation of peripheral blood specimens for involvement by TCLPDs, PCR and Southern blot TCR analysis have been the mainstay for establishing clonality. Flow cytometric immunophenotyping studies using antibodies to TCR-Vβ families also can be used to provide evidence of T-cell clonality. However, these flow cytometric assays largely have been used in a research setting owing to their complexity and the number of antibodies required. Reagents recently have become commercially available that potentially permit the incorporation of TCR-Vβ flow cytometric immunophenotyping into clinical laboratory practice. In our initial evaluation of one of these commercial kits, we found that the assay worked optimally in peripheral blood specimens less than 48 hours old. Sixty-five samples...
submitted for evaluation of potential involvement by a TCLPD met these criteria and were studied by conventional T-cell immunophenotyping, TCR-Vβ flow cytometric immunophenotyping, and molecular genetic TCR analysis.

Of the 65 analyzed cases, 36 had conventional T-cell immunophenotyping results that were normal or most consistent with a reactive process; 27 of these 36 showed no evidence of clonality by TCR-Vβ flow cytometric or molecular genetic studies. Of the remaining 9 cases, 4 had positive TCR PCR results, negative Southern blot results, and a normal T-cell immunophenotype; 3 had equivocal molecular genetic results; and 2 had PCR and Southern blot results that were consistent with clonality. All cases with an apparent discrepancy between the conventional T-cell phenotyping and molecular genetic results had abnormalities in TCR-Vβ flow cytometric immunophenotyping that in most instances (8 of 9 cases) was confined to the CD8+ T-cell subset.

Restriction of the T-cell repertoire has been described in a number of nonneoplastic states associated with prolonged stimulation of the immune system, including autoimmune disorders, viral infection, and normal aging.6,16-19 This phenomenon of repertoire restriction seems to occur primarily in the CD8+ T-cell compartment, and in this setting, clonal TCR gene rearrangements may be detected by varied molecular genetic techniques.7,20,21

The 2 cases with a normal or reactive conventional T-cell immunophenotype and clonal molecular genetic results occurred in patients with symptomatic HIV infection and an
autoimmune disorder (idiopathic thrombocytopenia purpura), respectively, disease states in which oligoclonal CD8+ T-cell expansions have been described.\textsuperscript{11,12,22} Comparison of these TCR-V\textsubscript{β} and molecular genetic results suggests that the latter might be sensitive enough to detect subtle perturbations in the CD8+ T-cell compartment that can be present in physiologic responses. In this setting, TCR-V\textsubscript{β} flow cytometric immunophenotyping, through its ability to provide a more detailed analysis of the composition of individual T-cell subsets, might aid in the interpretation of potentially diagnostically false-positive molecular genetic results.

T-cell clonality was demonstrated by TCR-V\textsubscript{β} flow cytometric immunophenotyping in 23 (79\%) of 29 cases in which a diagnosis of a TCLPD was made based on conventional T-cell immunophenotyping and molecular genetic methods. In addition, 3 of these cases were considered suggestive clonality by TCR-V\textsubscript{β} flow cytometric results owing to the overexpression of a single TCR-V\textsubscript{β} to near clonal levels or a predominance of cells that failed to react with any of the TCR-V\textsubscript{β} family antibodies, which cover approximately 70\% of the known TCR-V\textsubscript{β} specificities. There were 3 cases in which molecular genetic studies were positive but TCR-V\textsubscript{β} flow cytometric immunophenotyping failed to reveal evidence of clonality. Two of these cases were characterized by the presence of very low numbers of circulating abnormal cells, and the discrepancy between the molecular genetic and TCR-V\textsubscript{β} flow cytometric results likely reflects the greater sensitivity of molecular genetic methods. This result is not entirely unexpected. The determination of clonality by TCR-V\textsubscript{β} flow cytometric immunophenotyping requires a predominance of cells expressing a single TCR-V\textsubscript{β} compared with nonneoplastic cells present. Therefore, in situations in which there is diminution of the abnormal clone and reconstitution of the peripheral blood T-cell compartment by normal, nonclonal cells with diverse TCR-V\textsubscript{β} expression, the ability of TCR-V\textsubscript{β} flow cytometric immunophenotyping to discriminate the abnormal cell population is lost.

Analysis of TCR-V\textsubscript{β} expression through flow cytometric immunophenotyping provides a rapid tool for the detection of T-cell clonality that can be incorporated readily into the clinical laboratory through the advent of commercially available reagents. The rapid turnaround time for these studies,
the ease of T-cell subset analysis, and the potential to provide quantitative and qualitative results provide clear advantages over PCR and Southern blot TCR tests. However, TCR-β flow cytometric immunophenotyping seems to work best in limited specimen types and is not as sensitive as molecular genetic testing for detecting T-cell clonality owing to the comparative nature of results interpretation and the lack of antibody reagents to detect all TCR-β types.

To optimize the use of TCR-β flow cytometric and molecular genetic TCR assays, we use an algorithm to evaluate peripheral blood specimens for involvement by mature T-cell lineage lymphoproliferative disorders: Initial flow cytometric immunophenotyping studies are used with antibodies to conventional T cell– and NK cell–associated antigens, with use of 3- and 4-color reagents as needed to accurately delineate any distinctive cell population detected. If an abnormal T-cell population expressing αβ TCR heterodimer is detected by these studies, TCR-β flow cytometric immunophenotyping is performed; if this study provides unequivocal evidence of T-cell clonality in the appropriate T-cell subset, a diagnosis of a clonal T-cell disorder is made with further subclassification as appropriate. Cases with an abnormal T-cell phenotype that have equivocal or nonclonal results by TCR-β flow cytometric immunophenotyping are sent for molecular genetic testing, and a diagnosis is made based on correlation with the molecular results. This tiered approach to diagnostic testing permits the optimal use of laboratory resources and enhances the ability of the laboratory to provide a rapid, specific diagnosis.

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References


