To determine if circulating Sezary cells can be classified as reactive or neoplastic based on the ability to detect the presence or absence of clonal T-cell receptor beta chain (TCR-β) gene rearrangements by Southern blot analysis, we evaluated the peripheral blood of 25 patients: 11 patients with Sezary syndrome (SS), 11 with benign inflammatory dermatoses (BID), and three normal controls. Three of 11 patients with SS, with Sezary counts ranging from 14% to 52%, did not demonstrate any clonal TCR-β gene rearrangements in the peripheral blood, despite a TCR-β rearrangement by Southern blot analysis in the skin. Ten of 11 BID patients and all normal controls showed no evidence of a TCR-β gene rearrangement in the peripheral blood. However, one patient with psoriasis demonstrated a TCR-β gene rearrangement in the peripheral blood. The TCR-β gene rearrangement detected in this patient, confirmed with polymerase chain reaction (PCR) amplification of the TCR-γ gene rearrangement, did not correlate with the presence of circulating Sezary cells or the increased risk of neoplasia. Our results indicate that circulating Sezary cells may be monoclonal (neoplastic) or polyclonal (reactive), as defined by TCR gene rearrangement studies. Circulating Sezary cells in SS may be reactive in nature and not accurately reflect the actual tumor burden in the peripheral blood. The presence of circulating Sezary cells or the presence of a clone of cells defined by TCR-β gene rearrangement in the peripheral blood is not limited to neoplastic disease processes.

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extraction. In addition, from patients with SS, punch biopsies of skin were obtained, from which genomic DNA was extracted.

**Sezary counts.** Anticoagulated blood samples were centrifuged, and the white cell buffy coat was fixed in glutaraldehyde. After sectioning and dehydration, buffy coat sections were embedded in JB4 solution. Sections (1 μm) were prepared from the buffy coat and stained. The number of Sezary cells was recorded as the Sezary count; ie, the percentage of the mononuclear cells present with a cerebriform nucleus, as determined by one of us (C.J.) by light microscopy. The absolute number of circulating Sezary cells for each patient was calculated as the product of the Sezary count, the white blood cell count, and the percent lymphocytes recorded on the complete blood cell count differential.

**Southern blot and polymerase chain reaction (PCR) analysis.** Peripheral blood mononuclear cells were purified from patients' peripheral blood by Ficoll Hypaque sedimentation gradient centrifugation, snap-frozen in liquid nitrogen, and stored at −80°C. High-molecular-weight DNA was extracted from samples as previously described. DNA (7.5 μg) was digested to completion using two to five different restriction endonucleases (HindIII, EcoRI, EcoRV, BamHI). Electrophoresis (0.7% agarose gel), membrane transfer, prehybridization, hybridization, washing, and autoradiography were performed as previously described. A probe to the constant region of the TCR-β (Cβ) gene was labeled by nick translation using α-32P deoxyctydine triphosphate (dCTP). For one patient in the study, PCR amplification of peripheral blood lymphocyte genomic DNA was performed using TCR-Vγ and TCR-Jγ consensus oligonucleotide primers, and the amplification products were analyzed by denaturing gradient gel electrophoresis (DGGE), similar to the method of Wood et al.

**RESULTS**

Circulating Sezary cells were detected by morphology in one of three normal controls, 9 of 11 patients with BID, and in all 11 patients with SS (Fig 1). In normal controls and patients with BID, the Sezary count ranged from 0 to 13% of mononuclear cells, and the Sezary counts of the patients with SS ranged from 12% to 78%.

The results of TCR-β gene rearrangement studies by Southern blot analysis from patients with BID and SS and normal controls are summarized in Table 1. All 11 patients with SS demonstrated a TCR-β gene rearrangement on Southern blot analysis of skin. Figure 2 shows Southern blot analysis representative of two groups of SS: one demonstrating identical rearrangements in skin and blood (8 of 11), and the other demonstrating rearrangement only in the skin (3 of 11). The three patients that did not demonstrate a clonal TCR-β gene rearrangement on Southern blot analysis of the peripheral blood had Sezary counts ranging from 14% to 52% (Table 1), which should be well within the detection sensitivity of Southern blot analysis.

One of 11 patients with BID demonstrated a TCR-β gene rearrangement in the peripheral blood by Southern blot analysis. The patient had biopsy-proven plaque-type psoriasis (patient BID-1, Table 1) and a Sezary count of 2% on initial evaluation. Therapy at the time of evaluation included systemic retinoids; there was a history of prior phototherapy and methotrexate therapy. The Southern blot analysis on this patient demonstrated faint rearranged bands in the EcoRI and EcoRV digestions, corresponding to a rearrangement involving the Cβ1 locus, and did not demonstrate rearrangements in the HindIII and Xba I digestions, which would demonstrate a Cβ2 rearrangement (Fig 3). These findings suggested the presence of a relatively small population of monoclonal or oligoclonal T cells. When reexamined 9 months later, Southern blot analysis demonstrated the same result. However, the patient’s Sezary count was 0%. For confirmation, PCR/DGGE analysis of peripheral blood lymphocyte genomic DNA was also performed on this patient. At two different time points, the patient demonstrated a detectable monoclonal TCR-γ gene rearrangement (Fig 4), regardless of the presence or absence of detectable circulating Sezary cells. The patient shows no evidence of progression to cutaneous or systemic malignancy 2 years after his initial evaluation by Southern blot analysis. The 10 other patients with BID and the three normal control patients did not demonstrate detectable rearrangements in the peripheral blood by Southern blot analysis.

**DISCUSSION**

To determine if circulating Sezary cells as determined by morphology correlate with detection of T-cell clones with TCR-β gene rearrangements by Southern blot analysis, we evaluated patients with SS and unequivocal BID. In patients with SS, our results indicate that circulating Sezary cells can...
be heterogeneous. In 3 of the 11 patients with SS that we studied, circulating Sezary cells were interpreted as polyclonal or reactive based on their lack of a detectable TCR-β gene rearrangement by Southern blot analysis. These patients with SS had Sezary counts between 14% and 52%; therefore, our failure to detect rearrangements in these patients was not secondary to detection sensitivity. Our findings corroborate and extend previous genotypic studies of CTCL that demonstrate the absence of TCR gene rearrangements in the presence of circulating Sezary cells,16,21,23 These findings, along with morphologic, cytogenetic,13 and immunophenotypic studies,12,25 clearly demonstrate that polyclonal circulating Sezary cells may differ from the monoclonal malignant T cells infiltrating the skin and, therefore, may be termed reactive rather than neoplastic.16

Vonderheid et al13 demonstrated that circulating Sezary cells varied in size, and the presence of large-diameter Sezary cells correlated with the presence of polyploid malignant T cells. Meyer et al11 reported immunophenotypic heterogeneity among circulating Sezary cells in patients with CTCL when they demonstrated variable percentages of Sezary cells forming E-rosettes (CD2+). Furthermore, a recent study of patients with SS found that the absolute number of circulating malignant T cells, identified by TCR-variable region (TCR-Vδ) monoclonal antibodies, substantially exceeded those found with Sezary counts.23 This latter study implies that the malignant T-cell clone in CTCL may have a morphologic spectrum that may be modulated throughout the disease course, as well as within different tissue compartments.26 These results and our data indicate that circulating Sezary cells in CTCL may at various times represent different proportions of the clonal neoplastic and polyclonal reactive T cells.

Thus, the clinical and prognostic significance of the presence of circulating Sezary cells without a TCR gene rearrangement16,23 or the presence of a TCR gene rearrangement without detectable circulating Sezary cells in CTCL,24,27 needs to be more completely evaluated. In the study by Vonderheid et al,23 multivariate analysis showed that the proportion of large-diameter circulating Sezary cells was a major correlate with decreased survival, and the investigators reasoned that the increased proportions of large Sezary cells reflected the presence of cytogenetically abnormal malignant T cells in the blood. With respect to all circulating Sezary cells, detection of a TCR gene rearrangement may or may not have the same negative prognostic value as the proportion of large Sezary cells, i.e., the detection of the presence of the malignant clone in the peripheral blood may be associated with decreased survival. In one study, Bakels et al27 attempted to determine the prognostic significance of TCR-β gene rearrangements in the peripheral blood of patients with CTCL (mycosis fungoides). They identified 5 of 45 patients with varying clinical stages of mycosis fungoides (clinical SS was excluded) with TCR-β gene rearrangements in the peripheral blood as determined by Southern blot analy-

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**Table 1. Summary of Gene Rearrangement Studies in Normal Controls, Patients With BID, and Patients With SS**

<table>
<thead>
<tr>
<th>Diagnosis and Patient No.</th>
<th>Age (yr)</th>
<th>WBC Count (K/μL)</th>
<th>Sezary Cell Count</th>
<th>Southern Blot Analysis</th>
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<tr>
<td>Normal</td>
<td>37</td>
<td>5.8</td>
<td>0</td>
<td>G</td>
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<tr>
<td>Normal</td>
<td>25</td>
<td>6.6</td>
<td>0</td>
<td>G</td>
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<tr>
<td>Normal</td>
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<td>ND</td>
<td>1</td>
<td>G</td>
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<td>8.0/6.7†</td>
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<td>R/R†</td>
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Abbreviations: PBL, peripheral blood lymphocytes; G, germline; R, rearrangement; ND, not done (no differential available); ABS, absolute number of Sezary cells (see Materials and Methods).

* Southern blot analysis of TCR-β gene rearrangement.

† Repeat value (see Results).
with an unequivocal diagnosis of plaque-type psoriasis with no other underlying disease—a TCR-β gene rearrangement was detected. Repeat Southern blot analysis with multiple restriction enzyme digestions ensured that the rearranged bands were not artifactual (eg, partial digestion or plasmid contamination) and provided a means to identify the specific TCR-β locus (Cβ1 or Cβ2) involved in the rearrangement. Our results (Fig 3) demonstrate rearrangement involving the Cβ1 locus, which can represent the presence of either a monoclonal or oligoclonal population of T cells. However, TCR-γ PCR/DGGE analysis demonstrated a monoclonal gene rearrangement (Fig 4). These molecular findings were present both with and without detectable circulating Sezary cells, making the Sezary cells an unlikely source of the TCR gene rearrangements.

We speculate that our unexpected finding of a monoclonal TCR gene rearrangement in the peripheral blood of a psoriatic patient may reflect the role of the host immune response to pathogenic antigens or superantigens that may play an etiologic role in psoriasis. Leung et al studied two patients with acute exacerbations of psoriasis that appeared to be triggered by bacterial infection. Using monoclonal antibodies, they showed that skin biopsies from each patient demonstrated a restricted pattern of TCR-Vβ that corresponded to the Vβ pattern expected to be induced by the type of superantigen expressed during the infection. Using PCR and sequence analysis, Chang et al demonstrated that CD8+ T cells in psoriatic lesions preferentially use TCR Vβ3 and/or Vβ13.1 genes. The clonality of the Vβ sequence data suggested clonal expansion in situ in response to an antigen or antigens in the skin. Clonal dominance, or selective expansion of one (monoclonal) or more (oligoclonal) pathogenic T-cell clones with similar TCR-β gene rearrangements, has been well documented in nonmalignant clinical disorders such as allograft rejection, autoimmune diseases, and large granular lymphocytosis.

Posnett et al have also described clonal CD8+ T-cell populations in the blood of normal donors aged over 65 years. They speculated that these clones may represent cyto-

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**Fig 3.** Southern blot analysis of TCR-β gene rearrangement of a patient with psoriasis (patient BID-1) with circulating Sezary cells. Four endonuclease digestions of DNA extracted from peripheral blood lymphocytes (PBL) and controls (C) are demonstrated, with arrows representing clonal rearrangements. The endonuclease digestions of the TCR-β gene with EcoRI and EcoRV show Cβ-1 rearrangements, while digestions with HindIII and XbaI show Cβ-2 rearrangements. The analysis demonstrates faint rearranged bands in both EcoRI and EcoRV (arrows). Germline bands are indicated on the right by lines.

**Fig 4.** TCR-γ PCR/DGGE of a patient with psoriasis demonstrating TCR-β rearrangement by Southern blot analysis. Peripheral blood genomic DNA from two different time points (January 1993 and October 1993) was analyzed, using pairs of consensus primers: Vγ9 and Jγ1-2 (lanes 1 and 3) and Vγ1-8 and Jγ1-2 (lanes 2 and 4). An ethidium bromide-stained gel reveals the identical clonal bands (arrowheads) detected with Vγ9 and Jγ1-2 at both time points (lanes 1 and 3). Negative controls (peripheral blood lymphocytes from healthy young adults) are in lanes 5 and 6, and a positive control (Jurkat T-cell lymphoma cell line) is in lane 7.
toxic T cells with specificity for ubiquitous viruses or that they may be autoreactive cells, the T-cell equivalent of benign clonal gammopathies. The findings in our patient, at age 73 years, may be consistent with their observations. In 5 of 10 normal human individuals studied, Hingorani et al noted the clonal predominance of T-cell receptors within the CD8+CD45RO+ subset. They hypothesized that these cells may represent antigen-specific effector cells or populations of regulatory T cells.

One of the most important issues raised by our findings and those of others, is the proper interpretation of a TCR rearrangement in the peripheral blood in a benign or unknown setting. As noted above, monoclonality is not an exclusive feature of malignancy and can be seen in benign or reactive settings. Bakels et al analyzed the peripheral blood of 19 patients with benign exfoliative erythrodermas. One of these 19 patients (with the diagnosis of chronic dermatitis) demonstrated a clonal TCR-β gene rearrangement in the peripheral blood. No correlation with Sezary counts was provided. Wood et al reported evidence of dominant T-cell clonality by PCR/DDGE of skin samples in 6 of 105 non-CTCL/SS cases studied. These six cases consisted of four cases of nonspecific dermatitis and two cases of cutaneous lymphoid hyperplasia. They concluded that it will be important to determine the long-term risk of CTCL/SS among these patients with chronic clonal dermatitis and recommended close follow-up. The question, therefore, is whether the presence of TCR clonal rearrangements in benign settings indicates the existence or potential risk of malignancy or, perhaps, reflects an as yet undefined mechanism.

ACKNOWLEDGMENT

We are grateful to Dr Peter C. Nowell for his insightful comments and critical review of the manuscript.

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