The Clonal Nature of Circulating Sezary Cells

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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.

Sezary cells are lymphocytes with hyperconvoluted or cerebriform nuclear contours. Morphologically, they are characterized by a high nucleus-to-cytoplasm ratio, deep and narrow nuclear indentations, condensed chromat at the nuclear membrane, and cytoplasm poor in organelles.1 Sezary and Bouvrain2 first described these cells in 1938 in a patient with the clinical findings of erythroderma, intense pruritus, lymphadenopathy, and abnormal “monster,” hyperconvoluted mononuclear cells in the peripheral blood. This constellation was termed Sezary syndrome (SS). In 1968 using electron microscopy, Lutzner and Jordan3 confirmed the presence of cerebriform nuclei in the circulating cells of SS, which was later identified as a T-cell malignancy in the early 1970s by immunologic studies4 and classified as the leukemic variant of cutaneous T-cell lymphoma (CTCL).5 Cytogenic studies substantiated the neoplastic nature of Sezary cells in SS. As identical chromosomally abnormal clones were detected in patients’ skin and peripheral blood.6 Southern blot analysis of T-cell receptor beta chain (TCR-β) gene rearrangements7,8 further substantiated the clonal nature of CTCL and SS, demonstrating monoclonal rearrangements in the skin, lymph node, and peripheral blood.9,10

Although circulating Sezary cells have demonstrated clonality and are associated with SS, they are not specific to CTCL. Several studies have shown that cells with this distinctive morphology can also be detected in low percentages (less than 5%)12 in the peripheral blood of normal individuals and in percentages as high as 15%13 in the peripheral blood of individuals with benign inflammatory skin diseases (eg, psoriasis, atopic dermatitis).14 In addition, Flaxman et al12 reported the presence of Sezary cells in the cutaneous lesions of a variety of inflammatory dermatoses, solar-induced skin disorders (eg, solar keratoses and basal cell carcinoma), and vasculitis. The term reactive Sezary cell was introduced to discriminate nonneoplastic Sezary cells from neoplastic Sezary cells associated with malignant disease. ie, CTCL.15

The ability to distinguish neoplastic from reactive Sezary cells circulating in the peripheral blood is an important clinical issue affecting diagnosis and prognosis. In addition, it provides insight into the pathogenesis and clonal evolution of circulating Sezary cells and the disorders in which they are associated. Our goal was to determine if circulating Sezary cells can be classified as reactive or neoplastic based on the presence or absence of detection of clonal TCR-β rearrangements by Southern blot analysis. We evaluated two unequivocal disease groups: individuals with definitive clinical and/or histologic diagnoses of benign inflammatory dermatoses, and individuals with the clinicopathologic diagnosis of SS who had circulating Sezary cells in the peripheral blood and clonal TCR-β gene rearrangements in the skin.

MATERIALS AND METHODS

Patient selection and sample triage. Twenty-five individuals were analyzed in this study: 11 with benign inflammatory dermatoses (six cases of psoriasis, three cases of atopic dermatitis, two cases of erythrodemic drug reactions), 11 patients with SS, and three normal controls. All cases of benign inflammatory dermatoses (BID) had been previously categorized on clinical and/or histologic criteria. Patients with SS were receiving extracorporeal pheresis, with or without systemic interferon-alpha16,17 and all had histories of prior skin-directed therapies (topical mechlorethamine, phototherapy, or electron beam therapy). BID patients (atopic dermatitis/drug reaction) were being treated with topical or systemic corticosteroids. Psoriatic patients were being treated with phototherapy and/or systemic retinoids; some had histories of prior methotrexate therapy. After informed consent was given, blood was obtained via venipuncture from each patient, and portions of each sample were used for the following purposes: a complete blood cell count, a Sezary cell count, and peripheral blood lymphocyte isolation and genomic DNA.
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; i.e., 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, XbaI, 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 deoxycytidine 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 XbaI 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-\(\beta\) 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.\(^5\) 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.\(^6,22,34\) These findings, along with morphologic, cytogenetic,\(^3\) and immunophenotypic studies,\(^15\) 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 al\(^15\) 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 al\(^1\) 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

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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/(\mu)L)</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>0</td>
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<td>Normal</td>
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<td>—</td>
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<td>8.0/8.67t</td>
<td>2/0t</td>
<td>88/0t</td>
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<td>1</td>
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<td>ND</td>
<td>11</td>
<td>—</td>
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</table>

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-\(\beta\) gene rearrangement.

† Repeat value (see Results).

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**Fig 2.** Southern blot analysis of TCR-\(\beta\) gene rearrangements of two patients with SS with circulating Sezary cells. Clonal rearrangements (arrows) are detected in EcoRV endonuclease digests of DNA extracted from peripheral blood lymphocytes (PBL), skin (SKN), and controls (C). The analysis on the left demonstrates the identical rearrangement in PBL and SKN, while the analysis on the right demonstrates a rearrangement only in SKN, despite the presence of circulating Sezary cells. Germline bands are indicated on the right by lines.
sis. They did not correlate the molecular findings with circulating Sezary cells. Four of the five patients with a clone in the peripheral blood had histologic involvement of the lymph nodes. Only one patient of the five had limited skin disease without signs of extracutaneous involvement. Although this patient is reported to be alive and well with limited skin lesions 14 months after detection of the peripheral blood clone, no comparison is made with the 10 other patients in the study with similar stage disease but without detectable TCR-β gene rearrangements in the peripheral blood. This study is too limited to yield meaningful conclusions regarding the clinical significance of the molecular detection of disease in the peripheral blood in CTCL and in the absence of morphologic findings. Thus, further longitudinal studies are required.

In 1 of the 11 cases of BID that we evaluated—a patient 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.21 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.20 Leung et al20 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 al29 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,22 autoimmune diseases,20 and large granular lymphocytosis.31,32

Posnett et al33 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 Cβ1 rearrangements, while digestions with HindIII and Xba I show Cβ2 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: Vy9 and Jy1-2 (lanes 1 and 3) and Vy1-8 and Jy1-2 (lanes 2 and 4). An ethidium bromide-stained gel reveals the identical clonal bands (arrowheads) detected with Vy9 and Jy1-2 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.
totoxic 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.34 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,21,35,36 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.37 Bakels et al.35 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.31 reported evidence of dominant T-cell clonality by PCR/DGGE 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.

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