Decreased T-Cell Receptor Excision Circles in Cutaneous T-Cell Lymphoma

Kei-ichi Yamanaka,1 Nikhil Yawalkar,1 David A. Jones,1 Daniel Hurwitz,1 Katalin Ferenczi,1 Sara Eapen,2 and Thomas S. Kupper1

Abstract

Purpose: The T cell repertoire in patients with advanced cutaneous T cell lymphoma (CTCL) is significantly contracted despite the presence of relatively normal absolute numbers of T cells. We propose that many normal T cells were being lost in patients with CTCL, with the remaining normal T cells expanding clonally to fill the T cell compartment. T-cell receptor excision circles (TREC) form as a result of the initial gene rearrangement in naïve T cells. Although they are stable, they do not replicate and are subsequently diluted with the expansion of a population of T cells. Their concentration is therefore a measure of unexpanded naïve T cells relative to T cells that have undergone expansion.

Experimental Design: We analyzed TRECs from unfractionated peripheral blood T cells from 108 CTCL patients by quantitative PCR. In patients with obvious peripheral blood involvement, we also analyzed TRECs from clonal and nonclonal T cells.

Results: We found a decrease in the number of TRECs in peripheral blood of patients with CTCL at all stages of disease, and this decrease was proportional to the loss of complexity of the T cell repertoire as measured by complementarity-determining region 3 spectratyping. In patients with leukemic CTCL and a numerically expanded clone, we also found a significantly lower-than-expected number of TRECs in the nonclonal normal T cells.

Conclusions: We hypothesize that the nonmalignant T cells have proliferated to fill the empty T cell repertoire space left by the loss of other T cells, leading to diminished TRECs and loss of T-cell receptor diversity.

Primary cutaneous T cell lymphomas (CTCL) are a group of malignancies of memory T cells (1–3) that share a tendency for skin tropism. In early mycosis fungoides, the most common CTCL, the malignant cells are found almost exclusively in the epidermis and can usually be detected in the peripheral circulation only with highly sensitive PCR-based assays (4, 5). Progression of the disease could, in some cases, lead to an increase in the number of the malignant T cells in peripheral blood (3, 6), as well as in lymph nodes and nonlymphoid peripheral tissues (7). We previously reported that the T cell repertoire in patients with advanced CTCL is significantly contracted (8) despite the presence of relatively normal absolute numbers of T cells in blood (9, 10). These and other data led us to propose that many normal T cells were being lost in patients with CTCL, with the remaining normal T cells expanding clonally to fill the T cell compartment. This contraction of the T-cell receptor (TCR) repertoire may contribute to the immunosuppression and significant infection-related mortality that characterize advanced disease.

Recently, a new biomarker, known as T-cell receptor excision circles (TREC), has been reported to reflect recent thymic emigrants in human peripheral blood (11, 12). Signal joint ð Rec-J α (Rec-J) TRECs in the T-cell receptor ð locus are excised late in the course of thymic T cell development by the TCRA locus recombination process, which generates the repertoire of antigen-specific TCR α/β T cells (11). TRECs exist in cells as stable intracellular extrachromosomal circular excision products. These episomes do not replicate during mitosis and are thus diluted during subsequent T cell proliferation. The concentration of TRECs therefore measures the ratio of unexpanded naïve T cells relative to T cells that have undergone clonal expansion. Measurement of TRECs in human peripheral blood and thymus with the quantitative PCR can offer a tool to identify recent thymic emigrants and thus to estimate thymic output. The two other major known biological parameters affecting TREC levels are longevity of naïve T cells and the dilution of TREC by cell division (13).

If our hypothesis is correct regarding the deletion of nonmalignant T cells in CTCL, with the clonal expansion of surviving nonmalignant T cells to fill the T cell compartment, then we would predict that TREC levels should be decreased in the peripheral blood of patients with CTCL. In the present...
study, we analyzed TRECs in peripheral blood T cells from 108 patients with CTCL. We found a decreased copy number of TRECs in the blood of both early- and late-stage CTCL patients. The decrease in TREC levels was proportional to the degree of loss in complexity of the T cell repertoire, as measured by complementarity-determining region 3 (CDR3) spectratyping. We also found that patients with advanced disease and a dominant malignant clone had the lowest TREC levels. In these patients, even the nonmalignant T cell population showed reduced levels of TREC. The cause of the expansion of these nonmalignant apparently normal T cells is unknown, but we hypothesize that, in these patients, the remaining T cells have expanded to fill the empty “space” in the T cell compartment created by the loss of normal T cells and of their attendant diversity.

Materials and Methods

Patients and healthy donors. One hundred and eight patients with CTCL were recruited for this study from the Cutaneous Oncology Clinic at the Dana-Farber Cancer Institute after they provided informed consent. CTCL patients were classified according to the tumor-node-metastasis classification. The subject profiles are follows: stage I (41 males, 33 females; median age, 60 years, range 19-90), stage II (5 males, 2 females; median age, 69 years, range 31-82), stage III (9 males, 9 females; median age, 68 years, range 30-94), stage IV (3 males, 6 females; median age, 64 years, range 50-78), total (58 males, 50 females; median age, 61 years, range 19-94). Diagnosis was based on clinical criteria as well as on histologic and immunohistologic assessment of skin specimens. Blood specimens were also obtained from 33 healthy volunteers (19 males, 14 females; median age, 55, range 34-76) for comparison. All studies were approved by the Dana-Farber Cancer Institute, Institutional Review Board, under protocol 02016.

Preparation of purified CD3+ T cells. Peripheral blood mononuclear cells were isolated from heparinized venous blood obtained from patients with CTCL and healthy volunteers by density gradient centrifugation over Ficoll (Histopaque, Sigma, St. Louis, MO). CD3+ T cell populations were separated with immunomagnetic beads according to the manufacturer’s protocols (Miltenyi Biotec, Auburn, CA). Briefly, for CD3+ T cell selection, after 10 minutes of incubation with 20 μL of an antibody cocktail mixture, peripheral blood mononuclear cells were isolated for 15 minutes with 20 μL of magnetic beads per 10^7 cells. CD3+ T cells were then isolated from peripheral blood mononuclear cells by negative selection over MiniMACS separation LS columns. Sorted populations were analyzed by flow cytometry, and purity ranged between 96% and 99%.

Genomic DNA extraction. Genomic DNA was extracted from CD3+ T cells by means of the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer’s instructions. Prior to PCR amplification, DNA concentration in all samples was determined by UV spectrophotometry (Smartspec TM3000, Bio-Rad, Hercules, CA), and all samples typically had A_{260}/A_{280} ratios >1.6.

Quantification of T-cell receptor excision circles. Real-time PCR analysis was done to quantify the signal-joint β (Rec-β) and γ (Rec-γ) TRECs. Rec-J rearrangements occur late during T cell differentiation in the thymus, mostly after thymocyte expansion. Rec-J TRECs are considered to be a valid marker for these young T cells (14). We used a fluorescein-conjugated probe that hybridizes between the PCR primers. For PCR, the following primers and probes were used: sense, 5'-CGT CAG AAC GCT GAT CAA TCA AGA GCA GAA CAC A-3'; antisense, 5'-CAT CCC TTT CAA CCA CTT TGA CAC CTC TCT T-3'; and detection probe sequence, 5'-VIC-TTT TTG TAA AGC AGT TGC CCA CTC CTC TGC TCG ACG GTC A-TAMRA-3' (15, 16).

CDR3 spectratyping to identify contracted profiles and monoclonal peaks. For spectratyping analysis, total RNA was extracted from 3 × 10^6 CD3+ T cells using the Clontech RNA purification kit. Two to 5 μg of total RNA (A_{260}/A_{280} = 1.7-2.0) was reverse-transcribed using oligo-dT primers and Powerscript Reverse Transcriptase (Clontech). TCR β-variable (BV) segments were amplified with 1 of 26 BV subfamily-specific primers, as well as with CB primers recognizing both CB1 and CB2 regions. Sequences of BV 1 to 9, 11, 13 to 16, 18, and 20 primers were as in Choi et al. (18); those of CB and BV 10 primers, as in Geneve et al. (19); those of BV 22 and 24, as in Moss et al. (20); those of BV 12, as in Hall and Finn (21); those of BV 17 and 19, as in Bragado et al. (22); and those of BV 21 and 23, as in Hand et al. (23). BV subfamilies are numbered as in Wei et al. (24). PCR products were applied to a 5% polyacrylamide sequencing gel, and the size distribution of each fluorescent PCR product was determined by electrophoresis on an automated 377 DNA sequencer (ABI). With this technique, an amplified TCR BV subfamily migrates as a series of bands. Data were analyzed with GeneScan software (ABI) that assigns a size and peak area to the different PCR products.

Scoring of CDR3 profiles was done by determining the number of contracted BV CDR3 size profiles in each subject’s T cell CDR3 repertoire. Contracted profiles were defined as follows: oligoclonal (two to four peaks), monoclonal (one peak), or absent (no peaks detectable). The analysis was done by two different investigators in a blinded fashion (8).

Flow cytometric analyses. The presence or absence of a dominant clone in CTCL patients was determined by three-color flow cytometric analysis. Analysis was done on peripheral blood mononuclear cells using the following monoclonal antibodies to the TCR BV chain: PE-conjugated antibodies to BV 1, BV 2, BV 5.1, BV 5.2, BV 5.3, BV 7, BV 9, BV 11, BV 12, BV 13.1, BV 13.6, BV 14, BV 16, BV 17, BV 18, BV 20, BV 21.3, BV 22 (Immunotech/Beckman Coulter, Brea, CA); PE-conjugated antibodies to BV 3, BV 8, BV 23 (BD Bioscience, San Diego, CA); CD7 FITC (BD Bioscience), and CD4 PerCp (BD Bioscience). The isotype controls used were IgG1, PE, IgG2a, PE, IgG2b, PE, rat IgG1, PE (BD Pharmingen/Beckman Coulter), IgG2a, FITC, and IgG1, PerCp (Becton, Dickinson, Mansfield, MA). Cells were washed with PBS and then fixed with 1% paraformaldehyde; antibodies were used at a 1:100 dilution.
Immunophenotypic analysis of cells was done with a CellQuest flow cytometer (Becton Dickinson).

Dilution experiments. Normal CD3+ T cells from healthy volunteers were mixed with Jurkat cells (which do not have TRECs) in 11 combinations from 0% to 100% to simulate a hypothetical patient who had a mixture of clonal CTCL cells that are assumed to have expanded greatly and nonmalignant T cells. DNA was extracted and TREC levels were measured as described above.

Comparison of dilution experiment with patient data. TREC levels were compared between dominant clone–positive patients' actual data and predicted data extrapolated from dilution experiments. The predicted data was derived from the patient's percentage of clonal cells based on BV antibody flow cytometry measurements.

Purification of dominant clones and nonclonal cells. Dominant clones from CTCL patients were selected with antibodies to the appropriate TCR BV region as follows. First, CD3+ T cells were negatively selected with magnetic beads as described above. These cells were then incubated with PE-conjugated antibody to TCR BV targeted to the clonal BV (described above) and selected with anti-PE microbeads (Miltenyi Biotec). Nonclonal populations were passed through, and clonal cells were positively collected. The purity of the sorted population was analyzed by flow cytometry and ranged between 96% and 99%.

Statistical analysis. Linear regression models were fitted to the TREC data from all patients. The models included logarithmic values (to the base 10) of the TREC data as the dependent variable and age, gender, stage, number of monoclonal peaks, and contracted profiles as the independent variables. We also examined the correlations between log TREC levels and the number of monoclonal peaks and contracted profiles. The Wilcoxon-Mann-Whitney test was used to evaluate differences in TREC data between the normal cohort and cohorts with various mixtures of Jurkat and normal cells. The Bonferroni correction was used for multiple comparisons. We constructed 95% confidence intervals on the observed data from simulated experiments and checked if the actual TREC data was contained within them. We also constructed 95% confidence intervals on the CD3 T cells from 33 normal controls and checked if TREC data in nonclonal T cell population from 4 patients was contained within them.

Results

Analysis of T-cell receptor excision circles levels. TREC levels were measured in the unfractionated CD3+ T cell population in peripheral blood in 108 patients and 33 normal controls. A linear regression model was fitted to the logarithmic values of the TREC data with age, gender, and stage as covariates. The log-translated TREC data for all CTCL stages and the normal control population assume a Gaussian distribution (Fig. 1 and Table 1). TREC levels in CD3+ T cells were reduced significantly in patients at all stages of CTCL. No significant age- or gender-related differences were noted.

Having shown that TREC levels are lower in patients with CTCL, we next asked if there was a correlation between TREC levels and loss of CDR3 BV spectratype complexity. We recently showed that certain patients with stage I disease and most patients with higher stage disease had diminished TCR CDR3 BV spectratype complexity (8). Spectratypes were done on the same blood cell population for all patients whose TRECs were measured. Patients were judged to have contracted BV profiles if fewer than five peaks were present in a given BV family (normal, seven to nine peaks). We previously reported that single peaks could be seen in BV family spectratypes in CTCL patients, and that often patients had more than one BV family with single peaks (8). When sequenced, the PCR products from those single peaks always showed a single sequence, and thus we refer to them as monoclonal spectratypes. The number of monoclonal spectratype peaks for patients included in this study ranged from zero to eight. We then asked whether TREC values would decrease as the number of monoclonal peaks increased. To examine the

Table 1. Statistical analysis of TREC levels in CD3+ T cells in CTCL patients and normal donors

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>Parameter estimates</th>
<th>t Value</th>
<th>Pr &gt;</th>
<th>t</th>
</tr>
</thead>
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<tr>
<td>Intercept</td>
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<td>-1.24</td>
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<tr>
<td>Gender</td>
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<td>0.08510</td>
<td>0.12994</td>
<td>0.65</td>
<td>0.5137</td>
</tr>
<tr>
<td>Stage I versus Normal</td>
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<td>-1.28115</td>
<td>0.15932</td>
<td>-8.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stage II versus Normal</td>
<td>1</td>
<td>-1.42847</td>
<td>0.31738</td>
<td>-4.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stage III versus Normal</td>
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<td>-2.53462</td>
<td>0.22831</td>
<td>-11.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stage IV versus Normal</td>
<td>1</td>
<td>-2.09682</td>
<td>0.28884</td>
<td>-7.26</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
association between log TREC and the number of contracted BV spectratype profiles and monoclonal BV spectratype peaks, we looked at the Spearman’s correlation coefficient. This calculation was based on CTCL patients only and did not include the normal cohort of 33 patients.

The correlation between log TREC and the number of contracted profiles and monoclonal peaks was $-0.47$ and $-0.46$, respectively (Fig. 2A and B). There was a high positive correlation between the number of contracted BV spectratype profiles and monoclonal BV spectratype peaks (0.82), suggesting collinearity. Therefore, a linear model was fitted to the log TREC data, with the number of contracted BV spectratype profiles as the independent variable (Table 2).

The number of contracted profiles was a significant covariate. In this analysis, patients with five or greater contracted BV family profiles had TREC levels that were intermediate but still reduced. Patients with more than five monoclonal peaks had TREC values that were >100-fold lower than normal levels. Patients with three to five abnormal BV spectratypes had TREC values that were >100-fold lower than normal levels. Patients with one or no monoclonal peaks (Fig. 2B).

**Dilution experiments.** Patients with stage I and II CTCL and most patients with stage III disease did not have an expanded population of malignant T cells that was detectable by fluorescence-activated cell sorting analysis in peripheral blood. However, some individuals with stage III or IV disease did have a clone that represented >70% of their total T cell population. Because TREC are almost undetectable in malignant cells, we were concerned that this might cause TREC levels in unfractionated peripheral blood CD3+ T cells to be artificially low. Therefore, we did experiments to mimic the presence of clonal lymphoma cells in peripheral blood samples. Figure 3 shows the results of mixing T cells from a normal volunteer with Jurkat cells (which have no TREC) to simulate the blood of a hypothetical patient containing a mixture of nonmalignant T cells and a malignant clone that is assumed to have expanded greatly. The percentage on the $x$ axis in Fig. 3 refers to the Jurkat cells. Thus, at the 70% mark, 70% are Jurkat cells and 30% are normal CD3+ T cells. The two-sided Wilcoxon-Mann-Whitney test was used to assess the differences in TREC data between the normal cohort and the cohorts with various mixtures of Jurkat and normal cells. The Bonferroni correction was used for multiple comparisons at the 0.05 level of significance. Therefore, 11 comparisons were made: 0% versus normal, 70% versus normal, 75% versus normal, 80% versus normal, 85% versus normal, 90% versus normal, 92% versus normal, 94% versus normal, 96% versus normal, 98% versus normal, and 100% versus normal. The last six comparisons were significant, indicating that the overall measurement of TREC is not affected until the clonal population reaches 90% (Fig. 3).

**Comparison of Jurkat cell experiment with patient data.** We used a malignant clone mixed with normal CD3+ T cells to compare actual TREC data with predicted data extrapolated from Fig. 3. The predicted data was derived from the patient’s percentage of clonal cells based on BV antibody flow cytometry measurements. In all cases, the actual TREC levels in unfractionated peripheral blood were lower than levels that would be predicted if the nonclonal T cells were completely normal (Fig. 4). Table 3 presents the percentage of clonal cells, the means of actual TREC value, and 95% confidence intervals on the predicted data extrapolated from simulated experiments. There is no overlap between the means of the actual TREC values and the 95% confidence intervals on the predicted data extrapolated from simulated experiments. There are no overlap between the means of the actual TREC values and the 95% confidence intervals on the predicted data, when Jurkat cells were at 90%, 80%, 70%, 75%, and 94%.

**T-cell receptor excision circles data from malignant clone cells and nonclonal cells.** To validate that nonclonal cells had reduced TREC levels, we analyzed TREC values in these cell populations. In four of the above cases, we separated clonally expanded cells from nonclonal T cell populations using patients’ clone-specific TCR BV antibodies and magnetic beads, as described previously. In all four cases, the TREC values for clonal cells were extremely low, as would be expected.

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**Table 2. Association between TREC and the number of contracted profiles**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>t Value</th>
<th>Pr &gt;</th>
<th>Itl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>3.50346</td>
<td>0.29009</td>
<td>12.08</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Contracted profiles</td>
<td>1</td>
<td>-0.07568</td>
<td>0.03150</td>
<td>-2.40</td>
<td>0.0182</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 2. **A**, correlation between TREC and the number of contracted profiles on spectratype analysis in CTCL patients ($R = -0.470$). **B**, correlation between TREC and the number of monoclonal peaks on spectratype analysis in CTCL patients ($R = -0.460$).
TREC levels in clonal cells were 0.03% to 0.54% (average 0.19%) of those found in normal CD3+ T cells. However, and consistent with the data in Fig. 5, TREC levels were also lower in the nonclonal cell population of peripheral blood T cells from these patients than levels in normal CD3+ T cells. Table 4 presents the mean of TREC levels from four repeated observations from four patients’ nonclonal cells and 95% confidence intervals on the CD3+ T cells from 33 controls. There is no overlap between the means of the TREC levels in four patients and the 95% confidence intervals on the normal CD3+ T cells.

Discussion

New T cells are created in two different ways: thymic T lymphopoiesis, and postthymic expansion of T cells. New T cells created by the former process contain TRECs, and those created by the latter process do not. In this study, we showed that TREC levels are reduced in T cells from the peripheral blood of patients with CTCL. Median TREC levels in patients at all stages of disease, including stage I, were lower than normal, and were >100-fold lower than normal on average in patients with stage III disease. Although TREC levels are reported to decrease with age (25, 26), this was not a significant variable in the present study. Reduced TREC levels are also seen in other disease states, such as poorly controlled HIV infection (25, 26) and in certain immunodeficiency states (17). There is no evidence that our patients had either HIV infection or inherited immunodeficiency states, and we propose that lower TREC levels are an intrinsic feature of CTCL.

TREC levels are measured by PCR and are expressed as a ratio of TREC-specific DNA to 1 μg of T cell DNA. High TREC levels suggest that a T cell population contains a significant fraction of unexpanded naïve T cells, whereas lower TREC levels suggest a smaller fraction of unexpanded cells. A lower number of naïve cells could be due either to the preferential loss of such cells or to their clonal expansion, a process that would tend to dilute the TREC DNA as a fraction of total DNA in a cell population. We measured this dilution of TREC experimentally and showed that mitogenic stimulation of a T cell population produced a 10-fold decrease in TREC levels in 7 days and a 100-fold decrease in 14 days (data not shown). In patients with a dominant malignant clone that comprised >70% of total T cells, TREC levels would be expected to be lower than normal. This was indeed the case,

![Fig. 3. Dilution experiment. Normal CD3+ T cells from healthy volunteers were mixed with Jurkat cells, which do not have TRECs, to simulate a hypothetical patient with a mixture of clonal CTCL cells and normal CD3+ T cells. TREC data from this experiment indicate that the overall measurement of TREC level is not affected until the clonal population exceeds 90%.](image1)

![Fig. 4. Comparison of TREC data from Jurkat cell experiment with actual patients. We compared the data for actual patients with the same hypothetical situation of a malignant clone mixed with normal CD3+ T cells (taken from Fig. 3 and using the percentage of clonal cells in that patient based on BV flow cytometry measurements). In all cases, the data for the actual patients show decreased TREC levels compared with the levels predicted if the nonclonal T cells were completely normal.](image2)
but our data suggest that this cannot be accounted for simply by the presence of a large number of TREC-negative CTCL cells. Indeed, the nonmalignant cells have fewer TREC than would be expected from the analysis of a comparable number of normal T cells.

We previously reported a “collapse” of the T cell repertoire in patients with CTCL, and whereas this was most easily shown in patients with stage III and IV disease, it could be seen in a certain proportion of patients with stage I disease. In reviewing the spectratypes of these patients, we found that many BV families did not show the normal Gaussian distribution of CDR3 lengths. We routinely saw a reduction in the number of peaks (two to four, oligoclonal), single peaks (monoclonal), and no peaks (absent), sometimes in the same patient. Because the absolute number of T cells, as measured by fluorescence-activated cell sorting analysis with BV antibodies, was not reduced in these BV families, we hypothesized that normal T cells were being lost and that the remaining T cells in the same BV family were expanding clonally, a process that should result in such a spectratype profile. If this is true, the TREC levels in these BV families should also be markedly reduced. Although it is impractical to do a large number of TREC analyses on T cell subsets isolated with BV antibody, it is reasonable to predict that, in aggregate, TREC levels in CD3+ T cells in the peripheral blood of such patients would be lower than normal through simple dilution of TREC DNA after cell proliferation and that TREC levels would be even lower in patients with a large number of contracted profiles. A corollary to this would be that patients with multiple monoclonal peaks would have reduced TREC levels.

When we analyzed TREC levels as a function of contracted BV spectratypes in 108 patients with CTCL, we found a striking and statistically significant correlation between the number of contracted profiles and TREC levels, and patients with multiple contracted profiles had very low TREC levels. A corollary to this would be that patients with multiple monoclonal peaks would have reduced TREC levels.

When we analyzed TREC levels as a function of contracted BV spectratypes in 108 patients with CTCL, we found a striking and statistically significant correlation between the number of contracted profiles and TREC levels, and patients with multiple contracted profiles had very low TREC levels. Furthermore, because of a high positive correlation between the number of contracted profiles and monoclonal peaks (r = 0.82), TREC levels were strikingly correlated with the number of monoclonal peaks. These data support the hypothesis that patients with highly abnormal spectratypes have lost normal T cells and that other nonmalignant T cells have expanded clonally to fill the empty “space” in the T cell compartment created by this loss of T cells. Patients with poorly controlled HIV have both abnormal spectratypes and lower levels of TRECs, and both seem to normalize after highly active antiretroviral therapy (27, 28). This has been attributed to recovery of thymic function as viral loads decrease. We cannot rule out the possibility that thymic output is diminished in patients with CTCL. However, because the median age of our population was 61, baseline thymic function was already stable and diminished (25, 26). A further decrement in thymic function would not be expected to have such a profound effect on TREC levels and would not explain the correlation between TREC levels and spectratype findings.

In a subset of patients, we separated the expanded malignant clone from the putatively nonmalignant normal T cell population. Although the sample size was limited, the TREC level was clearly reduced in this nonmalignant normal T cell population (Fig. 5), again consistent with the interpretation that normal T cells had been lost and remaining cells had expanded clonally to fill the empty space. Neither

**Table 3.** Comparison of TREC data from Jurkat cell experiments with actual patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>% Clonal cells (% Jurkat cells)</th>
<th>Mean of actual TREC value</th>
<th>95% Confidence intervals for predicted TREC data</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>90</td>
<td>214.6273</td>
<td>1728.572-17239.8</td>
</tr>
<tr>
<td>B</td>
<td>80</td>
<td>45.01702</td>
<td>12372.79-106852</td>
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<tr>
<td>C</td>
<td>70</td>
<td>182.938</td>
<td>19451.41-67359.52</td>
</tr>
<tr>
<td>D</td>
<td>90</td>
<td>90.42937</td>
<td>1728.572-17239.8</td>
</tr>
<tr>
<td>E</td>
<td>90</td>
<td>821.2671</td>
<td>1728.572-17239.8</td>
</tr>
<tr>
<td>F</td>
<td>75</td>
<td>10612.52</td>
<td>41004-82647.05</td>
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<tr>
<td>G</td>
<td>94</td>
<td>9.03662</td>
<td>234.4466-1851.356</td>
</tr>
<tr>
<td>H</td>
<td>96</td>
<td>35.7389</td>
<td>31.66282-373.7292</td>
</tr>
</tbody>
</table>

NOTE: There is no overlap between the means of the actual TREC values and the 95% confidence intervals on the predicted data when Jurkat cells were at 90%, 80%, 70%, 75%, and 94%.

**Fig. 5.** TREC levels from patients’ nonclonal cells. The level of TRECs was reduced in the nonclonal cell population of the peripheral blood T cells from dominant clone–positive patients.
the mechanism by which this occurs nor the level at which the "set point" of T cell numbers is clear. One possibility is that the T cell compartment is homeostatically maintained at a certain size, a well-established phenomenon (29, 30). Because many patients with CTCL, even those with expanded monoclonal populations of Sezary cells, have normal absolute T lymphocyte counts, this implies that both normal and malignant T cells are subject to the same regulatory growth controls with regard to homeostasis as are the T cells in normal patients.

The cytokine responsible for maintaining homeostasis may well be interleukin-7. With regard to HIV infected patients, a low TREC number was reported to be associated with elevated serum interleukin-7 concentration (31, 32). We have analyzed plasma interleukin-7 levels in a larger number of CTCL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mean of TREC levels</th>
<th>95% Confidence Interval for CD3+ T cells from controls</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>1108.3</td>
<td>48813.98 – 187777.94</td>
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<tr>
<td>B</td>
<td>1179.475</td>
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<td>D</td>
<td>112.585</td>
<td>48813.98 – 187777.94</td>
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NOTE: There is no overlap between the means of the TREC levels in nonclonal T cell population from the four patients and the 95% confidence intervals on the CD3+ T cells from controls.

Acknowledgments

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References

21. Hall BL, Finn OJ. PCR-based analysis of the T cell...


Decreased T-Cell Receptor Excision Circles in Cutaneous T-Cell Lymphoma

Kei-ichi Yamanaka, Nikhil Yawalkar, David A. Jones, et al.


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