Monitoring the Decrease of Circulating Malignant T Cells in Cutaneous T-Cell Lymphoma During Photopheresis and Interferon Therapy

Katalin Ferenczi, MD; Nikhil Yawalkar, MD; David Jones, MD, PhD; Thomas S. Kupper, MD

**Background:** The prognosis of patients with stage IV cutaneous T-cell lymphoma (CTCL) is grim and therapeutic options are limited. Treatment of advanced-stage CTCL is aimed at suppressing the dominant T-cell clone, which is typically present in the skin, peripheral blood, and lymph nodes.

**Observations:** We detected the expansion of 1 T-cell clone expressing the T-cell receptor Vβ14 in the peripheral blood of a patient with stage IVA CTCL. Before initiation of combination therapy with photopheresis and low-dose interferon, the dominant T-cell clone represented 84% of the total T-cell population. After successful therapy, this clone showed a dramatic decrease to 6% of the T-cell population after 6 months of treatment. This reduction in the percentage of the malignant T-cell population in response to therapy was paralleled by clinical skin improvement from initial generalized erythroderma to undetectable skin disease.

**Conclusions:** This case demonstrates that response to combination treatment with photopheresis and low-dose interferon in patients with advanced CTCL may be accurately and quantitatively followed up by monitoring the percentage of the malignant T-cell clone (when identifiable) within the total circulating T-cell population by flow cytometry.

Arch Dermatol. 2003;139:909-913
cell lymphoma with lymph node involvement. The medical history was unremarkable. The patient’s only medication was fexofenadine hydrochloride. Findings on physical examination were remarkable for a nearly confluent, erythematous rash with minimal scaling and palpable adenopathy in the left axillary and inguinal area bilaterally. Histologic findings of an axillary lymph node were focally reminiscent of dermopathic lymphadenitis; however, atypical lymphocytes were identified with an immunophenotype similar to that of the cutaneous infiltrate. Thus, according to the TNM staging system, the patient’s stage was T4 N3 B2 and was classified as having stage IVA CTCL.

Computed tomographic scans of the chest and abdomen were unremarkable. Computed tomographic scans of the pelvis showed the presence of small retroperitoneal lymph nodes, measuring less than 1 cm.

Laboratory studies showed a normal white blood cell count, absolute CD4 count of 4869/µL; CD4/CD8 ratio of 14.8; 93% CD3, 89% CD4, and 6% CD8; and lactate dehydrogenase level of 750 U/L (normal range for this laboratory, 313–618 U/L).

Examination of a hematoxylin-eosin–stained skin biopsy specimen showed epidermotropism and a moderately dense upper and mid dermal lymphoid infiltrate composed of small to intermediate size lymphocytes with irregular nuclear outlines and condensed chromatin. The infiltrate had a perivascular and periadnexal distribution. Immunoperoxidase studies performed on paraffin sections showed the infiltrate to be composed predominantly of CD3+ cells with a predominance of CD4+ cells. There was partial loss of CD7 expression within the T-cell population.

Clonal rearrangement studies of the γ-TCR gene indicated a clonal population of lymphoid cells present within both the skin and the lymph node.

Photopheresis (on 2 consecutive days every 3 weeks) and low-dose interferon α therapy (3 million units 3 times weekly) were initiated.

### METHODS

**FLOW CYTOMETRY**

Peripheral-blood mononuclear cells were isolated from heparinized venous blood obtained from the patient and 4 healthy donors by means of density gradient centrifugation over Ficoll reagent (HISTOPAQUE; Sigma-Aldrich Corp, St Louis, Mo). Three-color flow cytometric analysis was performed with monoclonal antibodies against the TCR variable (Vβ) chain: unconjugated Vβ18; phycoerythrin (PE)-conjugated antibodies to Vβ1, Vβ2, Vβ5.1, Vβ5.2, Vβ7, Vβ9, Vβ11, Vβ12, Vβ13.1, Vβ13.6, Vβ14 (dominant T-cell clone), Vβ16, Vβ17, Vβ18, Vβ20, Vβ21.3, and Vβ22 (all from Immunotech, Westbrook, Me); PE-conjugated antibodies to Vβ3, Vβ8, and Vβ23 (BD Pharmingen, San Diego, Calif); cutaneous lymphocyte antigen fluorescein isothiocyanate (BD Pharmingen); and CD3 PerCp (peridinin chlorophyll protein) reagent (Becton, Dickinson and Company, San Jose, Calif). Isotype controls used were IgG1 PE, IgG2a PE, IgG2b PE, rat IgG fluorescein isothiocyanate (BD Pharmingen), rat IgG PE (Immunotech), and IgG1 PerCp (Becton, Dickinson and Company). After staining, cells were fixed with 1% paraformaldehyde and analyzed with a flow cytometer (FACScan).

**COMPLEMENTARY DETERMINING REGION 3 SIZE ANALYSIS OF TCR Vβ TRANSCRIPTS USING PCR**

Total RNA was extracted from healthy donors (n=6) and the patient’s peripheral-blood mononuclear cells with a reagent (Trizol; Life Technologies, Grand Island, NY). Complementary DNA was prepared by means of oligo-deoxynucleotidime primers and reverse transcriptase (Powerscript; Clontech, Palo Alto, Calif) according to the manufacturer’s instructions. The TCR Vβ14 segments were amplified with Cβ (fluorescent)-specific and Vβ14-specific primers as described previously. Diluted PCR products were applied to a 3% polyacrylamide sequencing gel, and the size distribution of each fluorescent PCR product was determined by electrophoresis on an automated DNA sequencer (ABI Prism 377; Applied Biosystems, Foster City, Calif). With this technique, an amplified TCR Vβ3 subfamily migrates as a series of bands, each one corresponding to a different complementary determining region 3 (CDR3) length. Data were analyzed by means of computer software (GeneScan; Applied Biosystems) that assigns a size and peak area to the different PCR products.

### RESULTS

We performed flow cytometric analysis of the patient’s peripheral-blood mononuclear cells by means of a panel of commercially available monoclonal antibodies against the human TCR Vβ chain covering more than 65% of the human TCR repertoire, as recently described in patients with Sezary syndrome. We were able to identify the expansion of T cells expressing the Vβ14 chain in the peripheral blood of this patient with stage IVA CTCL. Before initiation of the therapy, Vβ14+ T cells represented 84% of the total T-cell population (CD3+ gate; Figure 1A). Healthy control T cells from 4 donors were analyzed in a similar fashion, and the average percentage of the Vβ14+ T cells was 2.1%, ranging between 1% and 4%. An example of the Vβ14 expression in a healthy control is shown in Figure 1B. Flow cytometric analysis of peripheral-blood T cells isolated after 6 weeks of treatment showed a decrease in the percentage of malignant T cells from 84% to 62%, which continued to further decrease to only 15% of the total T-cell population after 18 weeks and 6% after 24 weeks (6 months) of photopheresis and interferon therapy (Figure 1A).

As shown in Figure 1A, malignant (Vβ14+) T cells showed diminished CD3 expression, an observation previously reported in a subset of patients with Sezary syndrome. The drop in the percentage of CD3low T cells during treatment correlated with the decrease in the percentage of the Vβ14+ T cells, and after 24 weeks the CD3low population disappeared (Figure 1A).

The decrease in the percentage of malignant T cells in the peripheral blood was accompanied by a drop in the absolute CD4 count from 4869/µL prior to treatment to 627/µL (reference range, 300–1300/µL) after 24 weeks of treatment. The CD4/CD8 ratio also decreased throughout the treatment (Table 1).

To further characterize the TCR Vβ14 gene segment of the expanded T-cell population, CDR3 spectratype analysis was performed. Spectratyping, a sensitive method to analyze the complexity of the T-cell repertoire, determines the distribution of lengths of the TCRβ chain. T cells from normal individuals usually demon-
In our patient with stage IVA CTCL who was receiving photopheresis and low-dose interferon α therapy, we were able to identify the malignant T-cell clone by means of flow cytometry and CDR3 spectratype size analysis. Using flow cytometry with a panel of antibodies to the Vβ chain, we identified the expansion of the dominant T-cell clone (Vβ14+) in the patient’s circulation and monitored the changes in the percentage of malignant T cells during successful combination treatment with photopheresis and interferon α. Further evidence that these Vβ14+ T cells represent a clonal expansion was obtained by CDR3 size analysis, which readily demonstrated a spectratype with a single peak before initiation of the therapy.

The results of the dramatic decrease in frequency of the malignant clone were also apparent in the Vβ14 spectratype profile of this patient. In contrast to the initially observed monoclonal profile, 4 distinct peaks were found after 24 weeks of treatment, indicating a persistent but strongly decreased Vβ14+ clone (at length 360) together with emerging detection of other presumably normal T cells with the use of Vβ14.

**Table 1. Changes in the CD4/CD8 Ratio Before and During Combination Therapy With Photopheresis and Low-Dose Interferon α**

<table>
<thead>
<tr>
<th>Treatment, wk</th>
<th>CD4/CD8 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment, wk</td>
<td>14.83</td>
</tr>
<tr>
<td>4</td>
<td>9.88</td>
</tr>
<tr>
<td>6</td>
<td>6.45</td>
</tr>
<tr>
<td>10</td>
<td>5.08</td>
</tr>
<tr>
<td>12</td>
<td>3.87</td>
</tr>
<tr>
<td>15</td>
<td>2.90</td>
</tr>
<tr>
<td>18</td>
<td>2.47</td>
</tr>
<tr>
<td>21</td>
<td>2.67</td>
</tr>
<tr>
<td>24</td>
<td>2.67</td>
</tr>
</tbody>
</table>

In Figure 2B, CDR3 size analysis in our patient’s blood before initiation of extracorporeal photopheresis and low-dose interferon α combination therapy showed a single peak for TCR Vβ14, which is highly suggestive of a monoclonal T-cell expansion. A spectratype profile consisting of 4 distinct peaks was found after 24 weeks of therapy, suggesting that the ratio of the only clone to normal Vβ14+ T cells had fallen dramatically.

Clinically, the patient presented with erythroderma involving more than 80% of the body surface, lymphadenopathy, and nearly intractable pruritus. After initiation of the combination therapy, the erythroderma gradually improved to scattered erythematous lesions involving less than 80% of body surface at 6 weeks, then to barely detectable disease at 18 weeks and complete resolution of the skin lesions at 24 weeks of therapy (Table 2). The gradual improvement and eventual clearing of skin lesions after 6 months of treatment was closely paralleled by the decrease in the percentage of malignant T cells and eventually the normalization of the Vβ14+ cells in the peripheral blood. Photopheresis and interferon and interferon α combination therapy also led to diminished lymphadenopathy.

**Comment**

Strate a highly diverse and polyclonal TCR repertoire with a typically gaussianlike distribution of CDR3 species of approximately 8 sizes. A representative spectratype from a normal individual is shown in Figure 2A. Contracted spectratypes consisting of 1 to 4 peaks suggest the presence of an oligoclonal or monoclonal T-cell population. As shown

**Figure 1.** Flow cytometry plots showing the percentage of Vβ14+ cells in the peripheral blood of a patient with stage IVA cutaneous T-cell lymphoma (CTCL) (A) before treatment and at 6, 18, and 24 weeks of therapy compared with an untreated control subject (B). The percentage of the Vβ14+ T cells in the patient and healthy control was analyzed by gating on the CD3+ population; the percentage of positive cells is indicated in the upper right quadrant. PE indicates phycoerythrin; PerCp, peridinin chlorophyll protein; and SSC, side scatter.

**Figure 2B.** Healthy Control
Methods commonly used to assess the efficacy of a treatment of patients with CTCL with peripheral-blood involvement are monitoring changes in the CD4+CD8 ratio and the percentage of CD4+CD7+ T cells. However, these methods are neither accurate nor specific for CTCL, since such changes can occur in other disease processes.13 Indeed, while an increased percentage of CD4+CD7+ T cells can be detected in a number of patients with advanced CTCL in other patients with CTCL with peripheral-blood involvement (such as in this patient), no expansion of T cells displaying this phenotype can be observed. The presence or absence of monoclonality in the circulation during the course of a treatment has been generally confirmed through TCR gene rearrangement studies, such as Southern blot or PCR. However, these nonquantitative studies cannot be used to determine exact percentages of malignant T cells within the total circulating T-cell population, limiting their utility for monitoring treatment efficacy. Flow cytometry has been recently used to determine the identity of the malignant T-cell clone in the peripheral blood of patients with CTCL.13 Although Vb typing is limited to families for whom specific antibodies are commercially available covering almost 70% of the human TCR repertoire, in a subset of patients with CTCL, the clonally expanded malignant population may not be identified.

Using flow cytometry, we were able to accurately measure the tumor burden in the blood of this patient with advanced-stage CTCL. The expanded Vβ14+ T-cell clone represented 84% of the total T-cell population before therapy and progressively diminished to 62% after 6 weeks of treatment. After 4.5 months, these cells had dropped to 15% and further to 6% after 6 months of photopheresis and low-dose interferon-α combination treatment. This progressive and dramatic decrease in the actual percentage of malignant T cells was paralleled by a gradual and eventually complete resolution of the erythroderma and pruritus. This case demonstrates the utility of direct identification of the malignant T-cell population in the peripheral blood of patients with advanced-stage CTCL, showing that monitoring the percentage of the malignant T-cell clone within the total T-cell population by flow cytometry can be an objective tool to accurately and quantitatively assess treatment efficacy. Analysis of the malignant T-cell population (when identifiable) might also allow prediction of response to a therapy and may be a useful tool in clinical trial designs in the future.

Accepted for publication November 20, 2002.

This work was supported by a Specialized Programs of Research Excellence (SPOReS) grant in skin cancer, National Cancer Institute, Bethesda, MD, and a Dermatology Foundation research grant, Evanston, Ill. Dr Yawalkar was supported by Novartis International AG, Basel, Switzerland, and Fondation René Touraine, Paris, France.

Corresponding author: Thomas S. Kupper, MD, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Boston, MA 02115 (e-mail: tskupper@rics.bwh.harvard.edu).

REFERENCES


**Archives Web Quiz Winner**

Congratulations to the winner of our April quiz, Fadi M. M. Hajjaj, MD, specialist dermatologist and venereologist, Abudhabi, United Arab Emirates. The correct answer to our April challenge was *annular elastolytic giant cell granuloma*. For a complete discussion of this case, see the Off-Center Fold section in the May ARCHIVES (Stratigos AJ, Antoniou C, Limas K, Katsambas AD. Large annular plaques on the trunk and arms. Arch Dermatol. 2003;139:657-662).

Be sure to visit the *Archives of Dermatology* World Wide Web site (http://www.archdermatol.com) to try your hand at the Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month's print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of the *The Art of JAMA II*.