Profound Loss of T Cell Receptor Repertoire Complexity in Cutaneous T Cell Lymphoma

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Abstract

Cutaneous T Cell Lymphoma is a malignancy of skin homing T cells. A major feature of CTCL is profound immunosuppression, such that patients with advanced mycosis fungoides or Sezary syndrome have been compared to patients with advanced HIV disease, and are susceptible to opportunistic infection. The etiology of this immunosuppression is unclear. We analyzed peripheral blood T cells of CTCL patients with Stage I-IV disease, using a sensitive beta-variable complementarity-determining region 3 spectratyping approach. Our data revealed a profound disruption of the complexity of the T cell repertoire, which was universally observed in patients with advanced disease (stage III and IV), and present in up to 50% of patients with early stage disease (stage I and II). In most patients, multiple monoclonal and oligoclonal CDR3 spectratype patterns in many different beta-variable families were seen. Equally striking was a reduction of normal T cells (as judged by absolute CD4 counts) across multiple beta-variable families. In general, CTCL spectratypes were reminiscent of advanced HIV spectratypes published elsewhere. Taken together, the data are most consistent with a global assault on the T cell repertoire in patients with CTCL, a process that can be observed even in early stage disease.

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Introduction

Primary Cutaneous T Cell Lymphomas (CTCL) are a heterogeneous group of malignancies of memory T cells that home to skin (1,2). Mycosis fungoides (MF) and Sezary syndrome (SS) comprise the majority of cases of CTCL. MF is characterized by erythematous patches, plaques or tumors on the skin, with or without lymph node involvement. SS is characterized by erythroderma and the presence of malignant T cells in peripheral blood (3). Transformed T cells in CTCL are typically CD4+, produce Th2 cytokines, and display skin homing markers such as CLA and CCR4 (4,5,6). An important diagnostic correlate in SS is the identification of a unique T cell receptor (TCR) rearrangement present in the circulating malignant T cells population, indicative of an expanded (relatively or absolutely) transformed T cell clone. Patients who present with early stage MF often survive for decades, while those with advanced disease (stage III or higher) have survival times measured in months (7,8). Death is often the result of bacterial sepsis that has been attributed to an incompletely characterized immunodeficiency in the context of a compromised integument (9). A Th2 cytokine profile of the malignant cells, decreased T cell responses to antigen, diminished T cell cytotoxicity, as well as to a relative paucity of normal T lymphocytes have all been suggested to contribute to this immunodeficiency (5,10,11).

We have frequently observed a decreased numbers of normal T cells in CTCL patients, particularly those with obvious peripheral blood involvement. This T lymphopenia is seen not only in SS patients where absolute T cell counts are high due to an expanded population of leukemic malignant T cells (11), but also in patients with normal total T lymphocyte counts. In many of these latter patients, a large percentage of T cell population actually represent an expanded malignant clone, so that the absolute counts of normal non-malignant T cells are greatly diminished. The cause of this relative lymphopenia is unknown, but suggests a systemic dysregulation of normal T cell production or survival. We designed the present study to examine the diversity of the T cell repertoire in CTCL. In particular, we performed quantitative analysis of peripheral blood T cell receptor beta-variable (BV) family expression by flow cytometry, and used complementarity-determining region 3 (CDR3) spectratype analysis of the same peripheral blood T cells using established BV primers. This latter technique is a measure of the complexity of the T cell repertoire (12,13), and is dramatically altered in diseases characterized by immunodeficiency, such as HIV infection (14), Omens syndrome (15), and idiopathic CD4 lymphopenia (16).

Our results indicate that there is a profound reduction in the complexity of the T cell repertoire in CTCL that in advanced disease is comparable to that seen in HIV infected patients. While uniformly present in patients with stage III and IV disease, this loss of Tcr complexity is also evident in some patients with very early disease (e.g., stage IA). Moreover, our results indicate that a dramatic decrease of normal T cells occurs in a non-random fashion. Taken together, these results are consistent with a process that is affecting the entire T cell population, leading to an oligoclonal dysplasia at the expense of the normal T cell repertoire.
Material and Methods

Patients and controls
Twenty patients with a histologically established diagnosis of CTCL were recruited from the Cutaneous Oncology Clinic at the Dana-Farber Cancer Institute after obtaining their informed consent. The characteristic of the patients are listed in Table 1. None of the patients were clinically infected at the time point of sampling. Six patients with psoriasis of moderate to severe severity (5 males, 1 female; mean age: 63 years, range 42-75), 1 patient with idiopathic erythroderma (m, 57 years) and 7 normal controls (3 male, 4 female; mean age: 58, range 40-70) were also recruited for comparison. The patients with psoriasis were all being treated with phototherapy and the patient with idiopathic erythrodermia had 6 cycles of extracorporeal photochemotherapy (ECP) and MTX 12.5 mg/weekly. The study was approved by the institutional ethics committee.

Preparation of cells/isolation of T cells
Peripheral blood mononuclear cells (PBMC) from patients and normal donors were isolated from heparinized venous blood by density gradient centrifugation over Ficoll (Histopaque®, Sigma, St. Louis, MO). T cells and their subsets (CD3+, CD4+, CD8+) were selected using immunomagnetic beads as described by the manufacturer (Miltenyi Biotec, Auburn, CA). The purity of the sorted population was analyzed by flow cytometry and ranged between 96–98 %.

Dilution experiments
In dilution experiments 1x10^6 T cells from a healthy donor (representing a polyclonal population) were mixed with increasing numbers of monoclonal Jurkat cells (0% to 97%).

CDR3 size spectratyping
Total RNA was extracted from 3-5x10^6 cells with Trizol reagent (Life Technologies, Grand Island, NY). 2-5 ug of total RNA (A260/A280 = 1.9-2.1) were reverse transcribed using oligo-dT primers and Powerscript™ RT (Clontech, Palo Alto, CA). TCR BV segments were amplified with one of 24 BV subfamily specific primers as well as CB primers recognizing both CB1 and C B2 regions as described previously (17). Sequences of BV 1-9, 11, 13-16, 18, 20 primers were as in Choi et al (18); of CB and BV 10 primers as in Genevee et al. (19); of BV 22, 24 as in Moss et al. (20); of BV 12 as in Hall and Finn (21); of BV 17 and 19 as in Bragado et al. (22); and 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 (Applied Biosystems). With this technique, an amplified TCR BV subfamily migrates as a series of bands, each one corresponding to a different CDR3 length separated from one another by 3 nucleotides. Data were analyzed by using the GeneScan software (Applied Biosystems) that assigns a size and peak area to the different PCR products.

Direct sequencing of PCR products
Amplified PCR products of selected patients were purified using the QIAquick® PCR purification kit (Qiagen, Valencia, CA). The purified products were directly sequenced in both directions with BV and CB specific primers using the ABI BigDye terminator sequencing kit (version 3) and a 3100 DNA sequencer (Applied Biosystems).

Flow cytometry
Three color flow cytometric analysis was performed on PBMC using the following monoclonal antibodies against the TCR BV chain: PE conjugated antibodies to: BV1, 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); CD3 PerCp and CD4 PerCp (BD Bioscience). Isotype controls used were: IgG1PE, IgG2a PE, IgG2b PE, Rat IgG1 PE (Immunotech/Beckman Coulter) and IgG1 PerCp (Becton Dickinson).

Integrated graphic representation by combining CDR3 spectratyping and the absolute numbers of BV+CD4+ T cells
Qualitative alterations of the TCR repertoire obtained by CDR3 spectratyping were combined with the quantity of specific BV+CD4+ T cells (i.e. absolute numbers of specific BV+CD4+ T cells) for each BV subfamily and plotted in form of a “landscape”. A similar analysis has been described previously (25). The area under the entire CDR3 profile is estimated as 100%. If several peaks are present in a CDR3 profile, the absolute numbers of BV+CD4+ T cells of this individual BV subfamily is segregated accordingly into several peaks. On the other hand if only one peak occurs, it will represent the absolute number of CD4+ T cells of that particular BV subfamily. The overall TCR BV repertoire is then plotted as a function of the 24 BV subfamilies (x-axis), CDR3 length distribution (z-axis) and the absolute number of BV+CD4+ T cells/mm3 (z-axis).

Scoring of CDR3 profiles and statistical analysis
Scoring of CDR3 profiles was performed by determining the number of contracted BV CDR3 size profile in each subject. Contracted profiles were defined as follows: oligoclonal (1-4 peaks), monoclonal (1 peak) or absent (no peaks detectable). The analysis was performed by 2 different investigators in a blinded fashion. Statistical analysis was performed using the Kruskal-Wallis nonparametric ANOVA test and subsequently by the Mann-Whitney U test (with the Bonferroni’s correction) for the unpaired samples. Correlation was tested using the Spearman rank analysis. A $p$ value of less than 0.05 was considered statistically significant.
Results

**CTCL patients with a malignant clonal expansion have a highly contracted TCR repertoire**

We analyzed peripheral blood from 20 patients with CTCL by FACS with a panel of antibodies specific for 21 different BV regions. Six patients were identified as having a clearly identifiable, numerically expanded population of a BV family consistent with a circulating malignant CTCL clone. The abundance of the expanded clone ranged from 73-96% of total CD4+ T cells analyzed, and in some (but not all) patients, the absolute CD4 count was elevated (Table I). Although there are no definite phenotypic markers which identify the malignant clone, a high percentage (>40%) of lymphocytes expressing a certain BV may represent a tentative criterion.

Peripheral blood T cells were isolated from each of these patients, and CDR3 BV spectratyping was performed. Fig. 1a shows a representative spectratype from peripheral blood T cells obtained from a normal volunteer. A Gaussian distribution of CDR3 lengths is observed in all BV families examined, indicating a highly diverse T cell repertoire across all BV families examined. In clear contrast, all six patients with identifiable circulating malignant T cell populations had strikingly abnormal spectratypes, and moreover these abnormalities involved multiple BV families. A representative example is shown in Figure 1b. In each of these six patients, an apparent clonal spectratype signature of a single peak could be identified in the BV family that was expanded by FACS analysis, indicating close correlation of these techniques (e.g., patient 5, Table 1 and Fig 1b). Direct sequencing of the PCR products from these single spectratype peaks revealed a single sequence, consistent with an expanded clonal population of T cells (data not shown).

Unexpectedly, however, apparent clonal populations could be also demonstrated in BV families that were not expanded by FACS analysis (figure IB). Additionally, spectratype patterns consisting of fewer than five peaks (which we will term oligoclonal), as well as apparent loss of BV families, were also noted. Absence of these BV families by spectratype analysis persisted even when an increased amount of DNA was used and 40 cycles of PCR were performed. Taken together, these data suggested that CTCL disease process affects much of the T cell population.

A trivial explanation for the aberrant spectratypes shown in Fig. 1b was the possibility that abundant cDNA reverse transcribed from a single expanded clone somehow interferes with the detection of normal polyclonal CDR3 cDNA, creating a false impression of loss of complexity. To test this possibility, increasing numbers of Jurkat T cell leukemia cells (BV8) were added to T cells from a normal donor, and spectratyping was performed on the mixture. Figs. 2a and 2b show the comparison of the normal spectratype to the spectratype of a mixture containing 97% Jurkat / 3% normal T cells. A normal background polyclonal spectratype appears in all BV families with the exception of BV8, which shows a single peak consistent an abundance of Jurkat cells. Not until Jurkat cells exceeded 99% of the total mixture did subtle artifactual abnormalities begin to emerge in other BV families (not shown). Further experiments showed that for a given BV family, the appearance of a single peak on a spectratype profile required a ratio of about 1:300 normal to clonal T cells; lower ratios yielded more than one peak (Fig. 2c). Thus, the ratio of an expanded clonal T cell to all other normal T cells in our patients would not be expected to interfere with the analysis of a normal spectratype. This indicates that the abnormalities we observed in multiple BV families were an intrinsic feature of CTCL and not a technical artifact.

The data above indicated that CTCL is not simply an expansion of a single malignant T cell clone against the background of a normal T cell repertoire, but rather a disease that affects the non-malignant T cell compartment as well. We next compared spectratypes of CD3 positive T cells from a total of 20 CTCL patients (including the six above; Table I) to those of seven normal donors, six patients with widespread psoriasis, and one patient with episodic idiopathic erythroderma who does not have CTCL. In a subset of these patients, CD3 T cells were separated into CD4 and CD8 subsets prior to analysis. In parallel, we examined peripheral blood T cells from these patients by flow cytometry. Absolute CD4 counts were reported for CTCL patients by the clinical laboratories at Brigham and Women’s Hospital.

**Patients with all stages of CTCL show markedly abnormal CDR3 spectratypes, while controls and psoriatics have normal spectratypes.**

There was a significant correlation of degree of spectratype abnormality with stage of CTCL (r_{spearman} = 0.69, P<0.05), with 100% of patients with stage III or higher showing markedly abnormal spectratypes (e.g., five or more contracted profiles). While some patients with stage I disease had normal spectratype profiles (not shown), other patients with stage I disease had significant abnormalities of
their T cell repertoire, seemingly out of proportion to their limited disease involvement (Fig. 1d and Fig. 3a). A similar significant \((r_{Spearman} = 0.54, p<0.05)\) correlation with stage could be seen when the number of single (monoclonal) peaks were enumerated in patients with different stages of CTCL (Fig. 3b). In data described above, we noted that a clone must be present in a roughly 300:1 ratio to all other T cells of the same BV to display as a single peak. While only 50% of patients with stage I and II disease showed monoclonal peaks, such single peaks could be detected in 100% of patients with stage III and IV disease. In contrast, no monoclonal peaks could be identified when spectratypes from normal volunteers or psoriatics were analyzed. In addition, a patient with idiopathic episodic erythroderma had an essentially normal spectratype (not shown). When the number of contracted profiles in spectratypes of CD3 (Figs. 3c and 3d), CD4, and CD8 (not shown) positive T cells from normal, psoriatic, and CTCL patients were compared, there was a significant difference between CTCL and both psoriatic and normals. There was no significant difference between normals and psoriatics compared in this fashion. Thus, persistent antigenic stimulation of the immune system per se (e.g., psoriasis) does not result in spectratype abnormalities in peripheral blood T cells. Analysis of both CD4 and CD8 T cells from patients with CTCL demonstrated spectratypes similar to those seen in CD3 T cells; therefore, the aberrant BV spectratypes were not a result of a single CD4 clone and multiple CD8 clones (not shown).

**BV families appear to be affected in a non-random fashion.**

We did not observe a random distribution of BV oligoclonal and monoclonal profiles across all BV families (Figs. 3e and 3f). For example, BV23 was abnormal (oligo or monoclonal) in 45% of the CTCL patients examined. When only stage III and IV patients were analyzed, abnormalities in BV23 were seen in 60% of patients. In contrast, certain BV families (e.g., BV 5.2, 6, 9, 20) were rarely affected. Some CTCL patients showed apparent deletions of BV families (e.g., BV 4 and 21 in Fig. 1c), even when higher amounts of cDNA and 40 cycles of PCR were used. Most frequently absent was BV 21, which was undetectable in 30% of CTCL patients, but was invariably present and normally complex in normal controls and patients with psoriasis.

Monoclonal BV spectratype peaks can be identified in patients who do not have expansion of the same BV family by FACS analysis, and such peaks correlate with a single clonal population of T cells by sequence analysis.

We have described above the correlation of single spectratype peaks with clonal expansions identified by FACS analysis and single sequences confirmed by sequence analysis in six patients. In an additional nine CTCL patients, spectratypes that showed at least one monoclonal BV family were obtained. While in 7 cases (in 5 different patients) this occurred in BV families for which we did not have a matching antibody, in 24 cases (in 10 different patients), FACS analysis of the BV family that showed a single peak by spectratype did not show relative expansion as compared to normal control values of BV. Sequencing of PCR products from the spectratype analysis of these single peaks again showed only a single sequence, consistent with a clonal population of T cells (data not shown).

**Analysis of clonal and oligoclonal populations in BV families that are not numerically expanded by FACS analysis suggests a widespread loss of normal T cells.**

By combining spectratype data with absolute CD4 counts and BV FACS analysis, we attempted to assess the nature of the apparent loss of repertoire complexity in CTCL, both qualitatively and quantitatively. Fig. 4a shows average absolute CD4 counts in each BV subfamily for stage I/II CTCL patients (n=7) and stage III/IV CTCL patients (n=10, without the numerically expanded clones), compared to normal reference values (26). For patients with stage III/IV disease, T cell numbers in all BV families were reduced with the exception of BV 23. An instructive example is shown in Fig. 4b. In this patient, the absolute number of CD4+BV 22+ cells is approximately 200 fold lower than normal values by FACS and cell count, yet a monoclonal pattern is shown in the BV22 spectratype in this patient. If a monoclonal peak indicates that the ratio of clonal to normal T cells is 300, then one can conclude that the number...
of normal, non-clonal T cells in this BV family is at least 200X300, or 60,000 fold reduced as compared to normal values. While imprecise, these data suggest that a significant loss of normal T cells is occurring across multiple BV families (at least in stage III/IV patients in a non random fashion associated with a relative clonal expansion of BV specific cells.

In order to generate an image that combines both CD4+ T cell depletion and reduced T cell repertoire complexity, we have generated topographic TCR “landscapes” that graphically demonstrate the loss of TCR repertoire complexity (Fig 5). A normal polyclonal diverse T cell repertoire is shown in Fig. 5a. A similarly complex landscape can be observed in psoriasis (Fig. 5b). In contrast, a CTCL patient with early stage disease is shown in (Fig. 5c). The loss of complexity is evident even at this stage of disease in the absence of an expanded clone by FACS. Even more striking is the landscape of a patient with stage III disease without an identifiable expanded clone by FACS analysis (Fig. 5d). In a stage III patient with a, numerically expanded clone identified by FACS, a single large peak appears to obscure the background (Fig. 5e). However, even when this clone is subtracted from the landscape, there is still very little background complexity (Fig. 5f).
Cancers in general, and lymphomas in particular, are considered to be the result of an accumulation of multiple genetic lesions in a single cell (27,28). Occasionally these genetic lesions are inherited, but most are acquired somatically, often as the result of exposure to genotoxic agents. In most cases, a single transformed cell and its clonal progeny are responsible for the growth of a malignant population of cancer cells. Typically, non-malignant cells of the same lineage do not appear altered. Certainly, the overwhelming majority of melanocytes are normal in patients with melanoma, and most lung epithelial cells appear normal (at least histologically) in lung cancer patients. In late stages of leukemias, and lymphomas, normal hematopoietic populations are altered (29,30), but this is typically ascribed to the mechanical and metabolic consequences of the overcrowding of anatomical compartments such as lymph nodes, peripheral blood, and bone marrow.

Our results with CTCL appear to be at odds with this paradigm. Rather than observing a single clonal population of malignant T cells against a background of normal T cells, we observed widespread and profound defects throughout the T cell repertoire. In our study, we examined T cell populations in CTCL patients at the level of BV families by FACS profile and CDR3 spectratype. There are 30 BV subfamilies, and we are able to analyze approximately 70% by FACS and 80% by CDR3 spectratype, giving us a reasonable working overview of the T cell repertoire. Our results show a global and profound reduction in the complexity of the T cell repertoire, to a degree previously seen in patients with advanced HIV disease or after myeloablative bone marrow transplantation (14,17). In all patients examined with stage III and stage IV disease, a significant fraction of the BV repertoire is strikingly abnormal. This is also true in roughly half of all patients examined with stage I and II disease. Within each BV family, the expected Gaussian distribution of CDR3 lengths is not seen; in contrast, single peaks or a ta more limited number of lengths are observed in spectratype profiles. When sequenced, these single peaks represent single CDR3 sequences, indicating a clone of T cells.

Clonal T cell populations are present in peripheral blood T cells in CTCL patients with all stages of disease, and these often involve multiple different BV families. This finding of multiple clones in some patients is consistent with other reported results (31). Most often, these BV families containing clones are not numerically expanded relative to the total CD4 count by FACS analysis. However, they do appear to be expanded at the expense of normal T cells within their BV family. Possible explanations for this are that a set of reactive clones is generated to an antigenic stimulus, or that there is a regulatory mechanism such that some T cells expand to fill the empty “space” created by the loss of other T cell populations. In addition, in several patients certain BV families are undetectable. While the numerically expanded clones in the six patients we analyzed did not favor any BV family, the BV spectratype abnormalities in our CTCL patients overall were non-random. For example, BV23 spectratypes were abnormal in 45% of all CTCL patients, and in 60% of Stage III and IV patients. BV 21 spectratypes were undetectable in 30% of all patients, and 40% of stage III and IV patients. Certain BV families nearly always showed normal spectratypes, including BV 5.2, 6, 9, and 20.

Prior medical therapy of patients does not seem to be a contributing factor to the spectratype abnormalities we observed. Several of our psoriasis patients had been on phototherapy or methotrexate, and their spectratypes were invariably normal. In addition, several of our stage I patients had had no therapy more potent than topical steroids. Finally, our patient with idiopathic erythroderma had been on photopheresis and methotrexate for many months, and had a completely normal spectratype. While we cannot rule out the possibility that cytotoxic agents like Denileukin Diftitox (Ontak) depleted additional populations of normal T cells, we consider it unlikely, as we did not observe further drops in absolute CD4 counts in patients on this drug. Additionally, we would expect Denileukin Diftitox to deplete normal T cells in a BV and clone independent fashion. The fact that we saw similar abnormalities both in patients who were treated and not treated with Denileukin Diftitox makes the possible contribution of this therapy unlikely. In summary, we could not attribute the significant changes in BV spectratypes observed to any prior therapy for this CTCL.

The reduction of TCR complexity in CTCL could be caused by a combination of different factors including specific interactions with the tumor cells (e.g. clonal expansion of T cells responding to CTCL associated antigens) and inappropriate activation.
and/or suppression of T cells (i.e. through activation induced apoptosis by cytokines or by the liberation of free unprocessed antigen). Persistent antigenic stimulation has been invoked as a possible oncogenic stimulus for T cells in CTCL (32). While antigenic stimuli may play some role in the abnormal spectratypes we see, our data with psoriasis patients strongly suggests that persistent antigenic stimulation per se does not result in significant spectratype abnormalities in peripheral blood T cells. Psoriasis is widely believed to be an autoimmune T cell mediated disease that is chronic and incurable (33). Many of our psoriasis patients had had their disease for many years, and some for decades. While occasional abnormal spectratypes were seen in some BV families, clonal patterns were never seen in these patients, and on balance the complexity of the T cell repertoire in psoriasis was comparable to that in normal individuals. In certain elderly patients with no evidence for disease, clonal expansions of single cells in a BV family yielding a single peak by spectratype have been noted. (34-36). Invariably, however, the remainder of BV spectratypes in such patients are normal. Typically, these benign clonal expansions occur predominantly in CD8 positive cells, and may be related to prior EBV or CMV viral infection.

We have shown that the spectratype patterns that we observed are not a dilutional artifact -- more than 97% of cells in a mixture of normal and leukemic cells can be clonal, and the patterns of unrelated BV families are normally complex. However, a significant amount of evidence has accumulated to suggest that the size of the T cell compartment in peripheral blood is tightly controlled under normal conditions (37). Depletion of large numbers of normal T cells leads to antigen independent proliferation and expansion of normal naïve T cells. Similarly, there is a process by which large numbers of normal T cells that have clonally expanded in response to antigen ultimately undergo apoptosis, leaving only a fraction of the population to survive as lon lived memory cells. Our data in CTCL patients are most consistent with a chronic process that results in widespread T cell dyscrasia, accompanied by T cell depletion. In this setting, surviving T cells may include those with a growth and survival advantage, and these “favored” cells may expand to occupy the depleted putative T cell niches. Such cells include not only the dominant malignant clone, but also other clones that may have accumulated genetic lesions that favor growth and/or survival without permitting unregulated autonomous expansion. Our spectratypes are in some ways similar to those of patients receiving T cell depleted bone marrow after myeloablative therapy for leukemia (17). In such patients, the small number of T cells transplanted along with hematopoietic progenitor cells appear to expand in an antigen independent fashion. Because so few T cells are transferred, they do not represent all possible BV families, or CDR3 lengths within those families. The spectratypes on peripheral blood of such patients after several months (when T cells have presumably expanded to fill empty niches) are quite similar to our stage III and IV CTCL patients.

The other clinical disorder where comparable spectratypes are observed is in advanced HIV infection (14). In such patients, wholesale depletion of CD4 positive cells is observed, but the spectratype patterns are similar to those of advanced stage CTCL patients. Losses in BV family complexity are seen across multiple families; however, whether this is due to compensatory expansion of certain clones of CD4+ cells with a growth advantage is unknown. In both HIV-1 patients and advanced stage CTCL patients (data not shown), the appearance of TREC DNA is very low (38). Since TREC’s are only seen in naïve T cells that have not clonally expanded, these data are consistent with compensatory proliferation of T cells attempting to fill the relatively empty T cell microenvironment in peripheral blood.

Finally, it is intriguing that only certain BV families appear to be abnormal in CTCL. As noted above, BV 23 is frequently affected, and BV 21 is frequently absent, in these patients. In contrast, BV 5.2, 6,9,20 are rarely if ever abnormal. The only known stimuli that affect T cells at the level of BV sequences are superantigens (39). Viral and bacterial superantigens have been reported to bind to and activate T cells via the BV segment of the T cell receptor, independent of the antigen recognition site. Superantigens from Staph. aureus have been implicated in oligoclonal T cell expansion in SS (40). We are unaware of any known bacterial superantigens that have affinity for BV 23 or 21, and the spectrum of viral superantigens is at present incomplete. Whether superantigens play a role in the compensatory T cell proliferation that we propose occurs in these patients is at present unknown.
It has been appreciated for many years that patients with CTCL, particularly those with advanced disease, are profoundly immunosuppressed. The cause of death in these patients is typically infection (9), and less frequently unrestrained tumor growth. In fact, CTCL patients have been compared clinically to patients with advanced HIV-1 with regards to their immune status. In the present study, we report that the T cell repertoire is dramatically abnormal in patients with CTCL. This is always the case in advanced disease, but can also be observed in patients with stage IA disease, including some with single patches involving less than 5% of their surface area. We do not have a clear-cut explanation for this dramatic loss of complexity, but feel that the presence of an agent that affects the T cell population as a whole is the most logical interpretation. Whether this is an undiscovered T cell tropic retrovirus, a different microbial pathogen, or a non-infectious process is at present unknown. Because of the striking similarities of CTCL spectratypes and HIV-1 spectratypes, we favor the first possibility. Previous reports implicating the lymphotropic retrovirus HTLV-1 in CTCL have so far remained conflicting (41,42), but involvement of a virus related to HTLV-1 remains a possibility. The precise nature of such a putative agent is the subject of intensive study.
References


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<td>IVA</td>
<td>no clone detectable</td>
<td>223</td>
<td>4.4</td>
<td>5-6 y</td>
<td>18 cycles ECP, IFN- , Bexarotene, Demileukin Diftitox</td>
</tr>
<tr>
<td>8/WS</td>
<td>m/61</td>
<td>IV</td>
<td>no clone detectable</td>
<td>1019</td>
<td>7.4</td>
<td>7 y</td>
<td>4 cycles of ECP, PDN, PUVA, IFN, total skin electron beam, Bexarotene 20mg</td>
</tr>
<tr>
<td>9/NP</td>
<td>m/74</td>
<td>III</td>
<td>no clone detectable</td>
<td>264</td>
<td>3.4</td>
<td>4 y</td>
<td>6 cycles of ECP, topical steroids narrow UV-B</td>
</tr>
<tr>
<td>10/AC</td>
<td>m/99</td>
<td>III</td>
<td>no clone detectable</td>
<td>1005</td>
<td>2.1</td>
<td>6 months</td>
<td>9 cycles ECP, PUVA</td>
</tr>
<tr>
<td>11/AF</td>
<td>m/72</td>
<td>IB</td>
<td>no clone detectable</td>
<td>701</td>
<td>7.2</td>
<td>4 y</td>
<td>9 cycles ECP, PUVA</td>
</tr>
<tr>
<td>12/MM</td>
<td>m/64</td>
<td>IB</td>
<td>no clone detectable</td>
<td>505</td>
<td>1.8</td>
<td>4 y</td>
<td>PUVA (20mg), topical steroids, nitrogen mustard</td>
</tr>
<tr>
<td>13/RS</td>
<td>m/73</td>
<td>IB</td>
<td>no clone detectable</td>
<td>624</td>
<td>1.75</td>
<td>3 y</td>
<td>Demileukin Diftitox, topical steroids, topical Calcipotriol</td>
</tr>
<tr>
<td>14/WG</td>
<td>m/70</td>
<td>IB</td>
<td>no clone detectable</td>
<td>253</td>
<td>3.4</td>
<td>8 y</td>
<td>PUVA, MTX, electron beam, ECP</td>
</tr>
<tr>
<td>15/LB</td>
<td>f/53</td>
<td>IB</td>
<td>no clone detectable</td>
<td>811</td>
<td>1.2</td>
<td>5 y</td>
<td>topical steroids and Tacrolimus</td>
</tr>
<tr>
<td>16/BB</td>
<td>m/77</td>
<td>IB</td>
<td>no clone detectable</td>
<td>443</td>
<td>1.1</td>
<td>3 y</td>
<td>topical steroids, topical steroids</td>
</tr>
<tr>
<td>17/VD</td>
<td>f/30</td>
<td>IA</td>
<td>no clone detectable</td>
<td>460</td>
<td>0.5</td>
<td>3 y</td>
<td>topical steroids, topical steroids</td>
</tr>
<tr>
<td>18/MR</td>
<td>m/44</td>
<td>IA</td>
<td>no clone detectable</td>
<td>819</td>
<td>4.0</td>
<td>5 y</td>
<td>topical steroids, MTX, topical steroids, topical steroids</td>
</tr>
<tr>
<td>19/NL</td>
<td>f/57</td>
<td>IA</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>10 y</td>
<td>No therapy in spontaneous remission since 10 years</td>
</tr>
<tr>
<td>20/EJ</td>
<td>f/60</td>
<td>IA</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>5 y</td>
<td>PUVA every 2 weeks, topical steroids</td>
</tr>
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</table>

Patients are grouped by identification of a numerically expanded clone (a) and without an identifiable expanded population (b). PDN: prednison, MTX; methorexate.
Figure legends

**Figure 1.** TCR BV CDR3 spectratyping profiles are strongly restricted in CTCL patients. 

*a*, CDR3 spectratypes of CD3+ T cells from a representative normal donor showing highly diverse profiles reflecting a heterogeneous TCR repertoire. 

*b*, a stage III CTCL patient with a numerically expanded malignant clone (BV20) demonstrating multiple monoclonal and oligoclonal profiles. 

*c and d*, Similarly contracted profiles as well as some apparent deletions (BV4, BV21) are also found in stage III patients without a numerically expanded clone and even in some IA patients. The y-axis represents relative fluorescence intensity (RFI) and the x-axis is CDR3 size (nucleotides).

**Figure 2.** TCR BV repertoire in normal T cells and cell mixtures of these with Jurkat cells. 

*a*, CDR3 spectratypes of T cells from a normal donor showing highly diverse profiles reflecting a heterogeneous TCR repertoire. 

*b*, Spectratype profiles of the cell mixtures containing 3% normal T cells and 97% Jurkat cells. As observed in A, polyclonal spectratype profiles were readily found across the other TCR BV subfamilies, even though 97% of the analyzed cells were composed of a clonal population (note the dominant peak in BV 8 representing Jurkat cells). 

*c*, Spectratype profiles of BV 8 from pure normal T cells (1) and normal T cells spiked with increasing numbers of Jurkat cells (2-7). A single dominant peak was observed at a ratio of normal BV 8+ T cells to Jurkat cells of 1:333.

**Figure 3.** Correlation of degree of spectratype abnormality with stage of disease. A significant correlation was found between the number of contracted (a) and monoclonal (b) spectratypes and the clinical stage. 

*c*, Quantification and statistical analysis of the number of contracted profiles in CTCL, psoriasis and normal donors. A contracted profile was defined as having 0-4 distinct peaks. 

CDR3 spectratyping was performed from CD3+ T cells. Statistically significant perturbations of the TCR repertoire of CTCL patients were found as compared to psoriasis and normal controls. Horizontal bars show mean values. Statistical analysis is indicated. 

**Figure 4.** BV+ CD4+ T cells are markedly depleted in CTCL. 

*a*, The absolute number of BV+CD4+ T cells was calculated by determining the percentage of BV+ CD4+ T cells by flow cytometry and obtaining absolute CD4 cell counts. The mean±SD of stage III/IV n=10, without the numerically expanded clones) and stage I/II CTCL patients (n=7) are shown in comparison to normal values for each BV subfamily. Normal values represent the average number of CD4+ T cells (1100/mm³, reference 26) and the normal mean percentage of BV+ CD4+ T cells in adult blood (mean ± SD are from a cohort of 85 normal specimens according to Beckman Coulter). With the exception of BV23 a markedly decreased number of BV+CD4+ T cells was observed particularly in stage III/IV CTCL patients. 

*b*, The combination of the reduction of absolute CD4+ values of specific BV subfamilies with a corresponding monoclonal spectratype profile reveals that a further depletion of BV22+CD4 T cells is actually present in this subfamily. An instructive example is shown.

**Figure 5.** Combination of a qualitative (CDR3 spectratyping) and quantitative (flow cytometry) analysis of the TCR repertoire. These graphs give a better visual assessment of the global reduction of the TCR diversity in CTCL. 

*a*, shows an overall diverse polyclonal
CDR3 profile from a representative normal donor. b, demonstrates a similarly divers profile from a patient with psoriasis. c, shows a CTCL patient with early stage disease. The loss of complexity is evident even at this stage of disease in the absence of an expanded clone by FACS d, shows a similarly restricted profile from a stage III CTCL patient without a numerically expanded clone. e, a highly restricted profile from a CTCL patient with a malignant clone and f, with this clone subtracted from the landscape, there is still very little background complexity. The x-axis displays the 24 BV subfamilies, the z-axis shows the CDR3 length distribution (in amino acids), and the y-axis indicates the absolute number of BV+CD4+ T cells/mm3.
Fig. 1

a. Normal donor
b. CTOL (Stage III, with malignant cells)
c. CTOL (Stage III)
d. CTOL (Stage IA)
Fig. 2

a  BV 1-24 (normal T cells = 100%)

b  BV 1-24 (normal T cells / Jkstet cells = 3%/97%)

c  BV 8
Fig. 3
Fig. 5

a Normal donor

b Psoriasis

c CTCL (stage IA)

d CTCL (stage III)

e CTCL (stage III) (with dominant clonal expansion)

f (graph shown without clone)
Profound loss of T cell receptor repertoire complexity in cutaneous T cell lymphoma

Nikhil Yawalker, Katalin Ferenczi, David A Jones, Keiichi Yamanaka, Ki-Young Suh, Sarah Sadat and Thomas S Kupper