$CD4^+/CD7^-$ T Cell Frequency and Polymerase Chain Reaction-Based Clonality Assay Correlate with Stage in Cutaneous T Cell Lymphomas

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In cutaneous T cell lymphomas, tumor cells can be found in skin and in other compartments. A precise definition of extracutaneous spread including blood involvement is necessary for staging and treatment design. We investigated peripheral blood in 51 patients with various types of cutaneous T cell lymphomas by the analysis of blood smears for Sézary cells, the $CD4^+/CD7^-$ T helper cell frequency in the peripheral blood by fluorescence activated cell sorter analysis and by polymerase chain reaction for the T cell receptor γ-chain followed by denaturing gradient gel electrophoresis. Eleven polymerase chain reaction products were sequenced. Thirty-five per cent of patients with stage Ia–IIb cutaneous T cell lymphomas presented a peripheral blood T cell clone. In patients with stage III–IVb cutaneous T cell lymphomas 75% were positive for clonality in the peripheral blood by polymerase chain reaction. Interestingly, three of 13 Sézary patients showed a TCR-γ joining region pseudogene (JγP1/JγP2) usage. $CD4^+/CD7^-$ cell counts were significantly higher in patients with advanced cutaneous T cell lymphomas than in patients with early cutaneous T cell lymphomas. There was a correlation between increased percentage of circulating $CD4^+/CD7^-$ cells and detection of clonality by polymerase chain reaction ($p = 0.001$). There was no significant correlation between the polymerase chain reaction data and the percentage of Sézary cells on blood smears. A significant correlation between $CD4^+/CD7^-$ cells and Sézary cells was found, however. Stepwise logistic regression analysis showed that the $CD4^+/CD7^-$ cell count and clonal T cell detection in peripheral blood are independently correlated with stage. The combination of both parameters gives more information than each one separately. In conclusion, our data indicate that fluorescence activated cell sorter analysis of peripheral blood and polymerase chain reaction-based clonality assays can improve the accuracy of staging investigations in cutaneous T cell lymphomas patients. ***Key words: $CD7^-$ antigen loss/clonality/cutaneous T cell lymphoma/mycosis fungoides/Sézary syndrome/T cell receptor. J Invest Dermatol 114:107–111, 2000***

Cutaneous T cell lymphomas (CTCL) are a group of clonal lymphoproliferative disorders originating in the skin. Tumor cells can be detected in skin and, especially in advanced stages, in other compartments (Bunn *et al*, 1980; Wood *et al*, 1994; Muche *et al*, 1997). Extracutaneous spread including blood involvement, is suggested to be associated with a poor prognosis (Weiss *et al*, 1989; van Bakels *et al*, 1992; Domman *et al*, 1996; Muche *et al*, 1997). Blood involvement can be recognized by various features:

1 Morphologically characteristic Sézary cells can be detected in the peripheral blood smear by light or electron microscopy. They have hyperconvoluted or cerebriform nuclei (Lutzner and Jordan, 1968; Burg *et al*, 1994).

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Abbreviations: CTCL, cutaneous T cell lymphoma; DGGE, denaturing gradient gel electrophoresis; SS, Sézary syndrome.

2 The $CD4^+/CD7^-$ T helper cell frequency in the peripheral blood lymphocytes can be determined by fluorescence activated cell sorter analysis. Clonal T cells in CTCL express CD2, CD3, CD4, CD5, and CD45RO, but lack CD45RA (Reinhold *et al*, 1993, 1997; Dummer *et al*, 1996b; Jakob *et al*, 1996). $CD4^+$cells with an abnormal phenotype (lack of CD2, CD5, or CD7) are expanded in some CTCL patients. Whereas the absence of CD2 or CD5 molecules is less common, CD7 is the most frequent and earliest antigen that is lost or expressed at very low levels (Bergman *et al*, 1998). The normal counterpart of $CD4^+/CD7^-$ cells occurs late in the immune response and is a stable differentiation state (Reinhold *et al*, 1996). There is a physiologic, age-dependent increase of $CD7^-$ cells (Kukel *et al*, 1994) and also in inflammatory dermatoses increased $CD4^+/CD7^-$ frequencies were observed (Harmon *et al*, 1996). A high proportion of Sézary cells is often associated with high $CD7^-$ counts in CTCL patients (Bogen *et al*, 1996; Harmon *et al*, 1996). The $CD4^+/CD7^-$, however, population does not directly represent the dominant T cell clone in many patients with CTCL, as clonal cells can be detected in the $CD4^+/CD7^+$ cell population as well (Dummer *et al*, 1999).

3 A predominant T cell clone in the blood can be detected by its identically rearranged T cell receptor (TCR), by southern blot for TCR-β or by polymerase chain reaction (PCR) amplification of segments of the TCR-γ (Bourguin *et al*, 1990; Wood *et al*, 1994; Meyer *et al*, 1997; Muche *et al*, 1997) followed by sequence-specific electropheresis, e.g., denaturing gradient gel electrophoresis.

We performed PCR/denaturing gradient gel electrophoresis (DGGE) for TCR in peripheral blood and determined the frequency of circulating $CD4^+/CD7^-$ T cells and Sézary cells. The relationship between patients' clinical stages and these data were evaluated.

MATERIALS AND METHODS

Patients Blood samples from 51 CTCL patients who are regularly seen at the lymphoma outpatient clinic of the Department of Dermatology, University Hospital Zürich, Switzerland were analyzed. Fifteen patients suffered from other diseases (seven inflammatory dermatoses, five cutaneous B cell lymphomas, two melanoma, one Kaposi's sarcoma). The CTCL were classified according the European Organization for Research and Treatment of Cancer classification (Willemze *et al*, 1997) and the TNM method was applied for staging (Bunn and Lamberg, 1997). The diagnoses of the CTCL patients were as follows: 21 mycosis fungoides, 13 Sézary syndrome (SS), five $CD30^+$ CTCL, four pleomorphic medium to large cell $CD30^-$ CTCL, one Granulomatous slack skin, one $CD8^+$ CTCL, one B cell rich CTCL, and five unclassified CTCL. The diagnosis was established by standard clinical and histologic criteria (Burg *et al*, 1997). Further diagnostic tools like chest X-ray, ultrasound, blood count, lymph node biopsies, and bone marrow aspiration were used to ascertain the stage. Thirty-six CTCL skin biopsies were available for molecular biology studies in addition.

Sample preparation Peripheral blood mononuclear cells (PBMC) pellets were prepared from heparinized blood by Ficoll-gradient separation (Pharmacia Biotech, Uppsala, Sweden). DNA from paraffin-embedded tissue samples was obtained by xylene extraction and then washed with ethanol. Pellets from both skin and blood samples were proteinase K digested. DNA was extracted twice with phenol/chloroform and once with chloroform and then precipitated with ethanol/ammonium acetate. The purified and dried DNA was resuspended in Tris-EDTA buffer before measuring of the nucleic acid concentration in each sample. Cell lines MyLa and Jurkat served as positive controls. MyLa, a human T cell line derived from mycosis fungoides, was a generous gift from Dr Keld Kaltoft, Institute of Human Genetics, Aarhus University, Aarhus, Denmark. Jurkat, a human T cell leukemia cell line, was obtained from ATCC (ATCC GIB 152).

PCR of TCR-γ For PCR amplification 1 μg of total DNA was suspended in 50 μl PCR solution containing 40 pM of each primer, 200 μM of dNTP, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.01% gelatine, and 2.5 units Taq DNA polymerase in a total volume of 50 μl. In a first step a PCR was performed with a primer set described by Bourguin *et al* (1990) and Wood *et al* (1994). Initially, 25 cycles using the "outer" TCR-γ variable region (Vγ)1–8 (5′ GAAGCTTCTAGCTTTCCTGTCTC 3′) and TCR-γ joining region (Jγ1/2 (5′ CGTCGACAACAAGTGTTGTTCCAC 3′) primers were performed, then 10 μl of the first round PCR product was added to 100 μl of fresh PCR buffer containing the "inner" set of Vγ1–8 (5′ CTCGAG-TGCGCTGCCTACAGAGAGG 3′) and Jγ1/2 (5′ GGATCCACT-GCCAAAGAGTTTCTT 3′) primers and another 20 cycles were performed. Cycle parameters in both rounds were: initial denaturation step at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 70°C for 40 s, followed by a terminal extension step at 72°C for 10 min. To amplify rearrangements involving Vγ9, the single Vγ9 primer (5′ GGAATTCCAAATTCTTGGTTTA 3′) replaced the outer and inner Vγ1–8 primer (Bourguin *et al*, 1990; Wood *et al*, 1994; Meyer *et al*, 1997). This protocol has been performed on all cases as a standard. Cases that demonstrated no clonality in both, blood and skin samples, when amplified with primers Vγ1–8, Vγ9, and Jγ1/2 were reamplified with consensus primers (expanded PCR) for variable regions Vγ10/11 (5′ CACTGGTACYGGCAGAAAC 3′; Muche *et al*, 1997), joining regions Jγ1/2 (5′CCCTCTATTACCTTGGAAATGTTG 3′; Taylor *et al*, 1991; modified), and JPγ1/2 (5′GAAGTTACTATGAGCYTAGTCCCTT 3′; Greiner *et al*, 1995). With these primers a single round PCR was performed with the following thermocycling conditions: initial denaturation step at 94°C for 4 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, followed by a terminal extension step at 72°C for 5 min. The reactions were performed in a Thermal Cycler 9600 (Perkin-Elmer, Küsnacht, Switzerland). Primers were obtained from Microsynth (Balgach, Switzerland).

DGGE This was performed with the D Gene System (Bio-Rad, Hercules, CA). The PCR products were precipitated and resuspended in 10 μl of deionized water, heat-denatured at 95°C and then reannealed for 30 min at 30°C. The samples were loaded on a 6.5% polyacrylamide gel containing gradients of 30–60% urea/formamide and electrophoresed at 60°C for 6 h at 150 V. The ethidium bromide-stained gel was photographed under ultraviolet light (Bourguin *et al*, 1990; Wood *et al*, 1994; Meyer *et al*, 1997) (**Fig 1**).

The sensitivity of the PCR/DGGE analyses was estimated by gradually diluting cells from the Jurkat cell line (rearranged Vγ8 and Vγ11) with peripheral blood lymphocytes of a healthy donor as polyclonal background. The serially diluted Jurkat cells (1:5, 1:10, 1:100, 1:1000) were pelleted and DNA was extracted as described above. On the DGGE gel a clonal band was still clearly detectable when the Jurkat cells represented 10% of the cells in the sample.

Sequencing PCR products that formed a sharp band on the DGGE gel were cut out, eluted from the gel and then reamplified with the same primers as before to increase the amount of DNA. The PCR product was purified with the QIAquick PCR purification kit (Qiagen, Basel, Switzerland) and then sequenced at Microsynth (Balgach, Switzerland).

Flow cytometry The PBMC from 47 CTCL patients were screened with monoclonal antibodies against CD4 and CD7 by two-color fluorescence activated cell sorter analysis (Becton Dickinson, San Jose, CA) (Dummer *et al*, 1999). [Antibodies from T cell Diagnostics (Woburn, MA) and Immunotech (Westbrook, ME).]

Sézary cell count The portion of Sézary cells was determined by light microscopic analysis of peripheral blood smears from 13 patients with SS by a hematologist.

Statistical methods Differences between groups with regard to continuous data were tested by the Mann–Whitney test and figured using boxplots. The "box" indicates the range from the 25th to the 75th percentile, the horizontal line in the box is the median. The under and upper horizontal lines (whiskers) specify the 10% and 90% percentiles, "o" are extreme values. Correlations of continuous data were analyzed using linear regression. Univariate and stepwise logistic regressions were performed to assess predictors of stage.

RESULTS

PCR/DGGE analysis of skin and blood samples The PCR of the control patients did not detect a T cell clone in the blood samples.

PCR of CTCL patients Stage Ia–IIb (group1): In 18 of 20 patients (90%) a clonal T cell population was detected in skin biopsy and in seven patients (35%) in the peripheral blood. All seven patients with early blood involvement responded well to therapy during a stage-adapted therapy with psoralen ultraviolet A alone or in combination with interferon (Dummer *et al*, 1996a). The follow-up period after the PCR analysis was between 1 and 3 y. All patients remained in the same stage at time of the data evaluation as they were when the PCR analysis had been performed. Two patients were in stage Ia, two in stage Ib, two patients in stage IIa, and one in stage IIb. Two of these seven patients suffered from pleomorphic $CD30^-$ CTCL. They were both in stage I and presented neither lymph node involvement nor other signs of extracutaneous disease besides the circulating clonal cells.

Stage III to IVb (group 2) In 12 of 16 patients (75%) a clonal T cell population was detected in both blood and skin. In four additional patients, only PBMC were available. All were positive for a T cell clone (**Table I**).

Expanded PCR results *versus* standard PCR results In seven of 20 patients with advanced CTCL (group 2), clonality was not detected with the standard primer panel. In four of these seven

patients the clone was detected in skin and blood samples with primers Vγ10/11 and JPγ1/2. Three of 13 patients (23%) with SS showed a JγP1/2 segment usage. The sequences of rearrangements are shown in **Table II**.

In stages Ia–IIb 18 of 18 detected clones showed a rearrangement involving the segments Vγ1–9 and Jγ1/2. In stage III–IVb 13 of 17 clones (76.5%) showed a rearrangement with segments Vγ1–9 and Jγ1/2. Three clones (17.6%) used the JγP1/2 segment for the rearrangement whereas one clone was using the Vγ10 segment.

Circulating $CD4^+/CD7^-$ T cells and stage $CD4^+/CD7^-$ counts were significantly higher ($p = 0.0006$) in group 2 (patients in stage III–IVb) than in group 1 (patients in stage Ia–IIb). Two-color fluorescence activated cell sorter analysis demonstrated that CTCL patients had a mean of 26.1% $CD4^+/CD7^-$ cells (median, 14.2%; SD, 25.4%; range, 4.9–90.7%). Group 1 patients had a mean of 13.7% $CD4^+/CD7^-$ cells (median, 12.6%; SD, 5.8%; range, 4.9–30%). Group 2 patients had a mean of 44.3% $CD4^+/CD7^-$ cells (median, 41.1%; SD, 31.7%; range, 5.3–90.7%). (**Fig 2**).

There is a tendency towards higher $CD4^+/CD7^-$ T cell counts in SS compared with mycosis fungoides. In stage IVa mean $CD4^+/CD7^-$ counts were 46.53% (SD, 26.68%, $n = 4$) in SS patients and 13.0% (SD, 5.52%, $n = 2$) in mycosis fungoides patients. The $CD4^+/CD7^-$ T cell counts in patients with CD30 positive lymphomas were: in stage Ia (1 patient) 17%, in stage Ib (2 patients) 10.9% and 13.6%, in stage IIb (1 patient) 23.2%, and in stage IVa (1 patient) 11.4%. The $CD4^+/CD7^-$ count in the single case of a $CD8^+$ cutaneous lymphoma was 13%.

Circulating $CD4^+/CD7^-$ T cells and clonality in PBMC Patients with clonal cells in their peripheral blood had a significantly increased percentage of circulating $CD4^+/CD7^-$ cells ($p = 0.001$). Patients with a negative PCR result had a mean of 13.4% $CD4^+/CD7^-$ cells (median, 12.1%; SD, 5.7%; range 4.9–30%). Patients with a positive PCR result had a mean of 41.8% $CD4^+/CD7^-$ cells (median, 30.0%; SD, 31.2%; range, 5.3–90.7%) (**Fig 3**).

Sézary cells and clonality in PBMC There was no significant correlation between the PCR result and percentage of Sézary cells ($p = 0.27$). Nevertheless a tendency towards higher Sézary cell rates was observed in blood in which clonal T cells were detected. Mean Sézary cell percentage of patients with negative PCR result was 16.7% (SD 28.9%; range 0–50%). Mean Sézary cell percentage of patients with positive PCR result was 39.6% (SD 36.6%; range 0–90%).

Circulating $CD4^+/CD7^-$ T cells and Sézary cell count Linear regression analysis showed a significant correlation between $CD4^+/CD7^-$ cells and Sézary cells ($p = 0.03$; $R^2 = 0.36$; regression plot in **Fig 4**).

Relationship of PCR positivity in PBMC and stage Logistic regression analysis showed that the relative risk of a patient to be in the stage group 2 (stage III–IVb) is significantly influenced by the PCR-based detection of clonal cells in the peripheral blood ($p = 0.0003$). The odds ratio was 13.7 which means that a patient with a positive PCR result is about 14 times more likely to be in the stage group 2.

Table I. Clonality detection[a]

Stage	Blood sample	Skin sample
Ia	2/7	3/4
Ib	2/10	6/7
IIa	2/9	6/6
IIb	1/5	3/3
III	8/10	6/9
IVa	5/7	4/5
IVb	3/3	2/2

[a]Number of samples where a T cell clone was detected *versus* total number of samples.

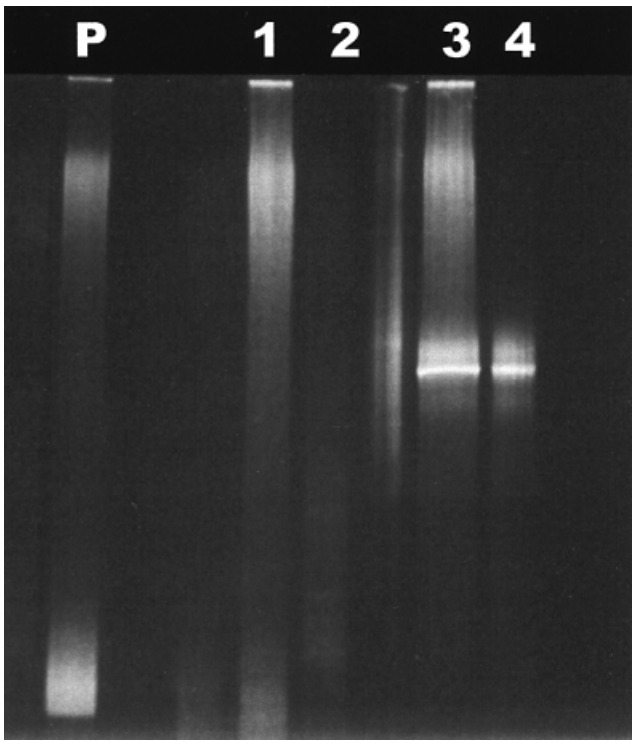

Figure 1. PCR/DGGE from patient samples, negative controls, and positive control. *Lanes 1* and *2* are skin and blood samples from a healthy donor. *Lane 3* is the blood sample and *lane 4* the skin sample of a patient with SS. The same mobility of the two bands suggests the same T cell clone in both samples, which was confirmed by sequencing. Lane "P" is the positive control.

Table II. Sequencing results

Diag.	Pat.	Sample	V segment	N region	J segment
SS III	Xa	Blood	V8 TCTATTACTGTGCCACC	AA	TGGTTGGTTCAAGATA JP1
		Skin	V8 TCTATTACTGTGCCACC	AA	TGGTTGGTTCAAGATA JP1
CTCL III	Ra	Blood	V10 ACTACTGTGCTGCGTGGG	GCTCCTCC	ATAAGAAACTCTTTGGCA J2
		Skin	V10 ACTACTGTGCTGCGTGGG	GCTCCTCC	ATAAGAAACTCTTTGGCA J2
SS IVb	Wi	Blood	V10 ACTACTGTGCTGCGT	AGAA	ATACCACTGGTTGGTTCAA JP1
		Skin	V10 ACTACTGTGCTGCGT	AGAA	ATACCACTGGTTGGTTCAA JP1
SS III	Eg	Blood	V4 GAGTCTATTACTGTGCC	TCCGCCTTA	AGTGATTGGATCAAGACG JP2
		Skin	V4 GAGTCTATTACTGTGCC	TCCGCCTTA	AGTGATTGGATCAAGACG JP2

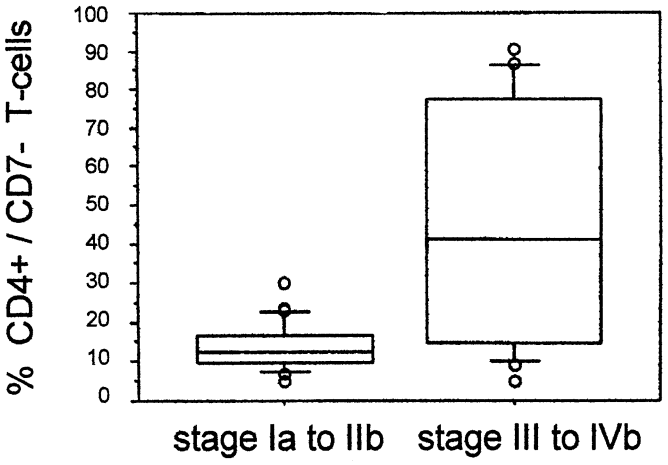

Figure 2. Percentage of $CD4^+/CD7^-$ cells in the two patient groups (stage Ia to stage IIb and stage III to stage IVb).

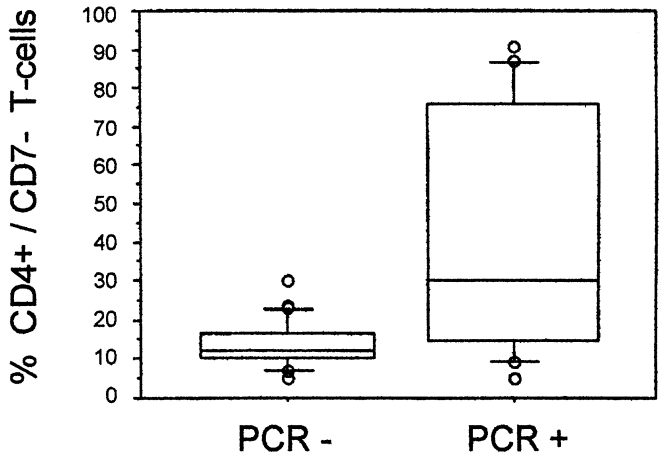

Figure 3. Percentage of $CD4^+/CD7^-$ cells in patients without or with detection of clonal cells in the peripheral blood.

Relationship between circulating $CD4^+/CD7^-$ T cells and stage Logistic regression analysis showed that the relative risk of a patient to be in the stage group 2 (stage III–IVb) is significantly influenced by the $CD4^+/CD7^-$ cell ratio in the peripheral blood ($p = 0.0013$). The odds ratio was 3.8 which means that if the $CD4^+/CD7^-$ ratio is doubled, a patient is four times more likely to be in the stage group 2. (From the $CD4^+/CD7^-$ values the dual logarithm was taken prior to the analysis to avoid an inappropriate risk increase in the high $CD4^+/CD7^-$ value range.)

Stepwise logistic regression analysis $CD4^+/CD7^-$ cell count and clonal cell detection in the peripheral blood have both a significantly ($p = 0.03$) independent influence on the stage. They are both strong stage markers. Including both parameters gives more information about the stage than to consider each separately [Odds ratios (OR): log2 ($CD4^+/CD7^-$): OR = 2.7; PCR result, OR = 5.9].

DISCUSSION

To investigate peripheral blood involvement in CTCL, we compared blood smears, the $CD4^+/CD7^-$ count and the PCR-based detection of circulating clonal cells.

A T cell clone can be identified by its identically rearranged TCR. The TCR is expressed on the cell surface of T lymphocytes as an αβ- or γδ-heterodimer and is responsible for antigen recognition. Ninety-eight to 99% of human T cells are αβ T

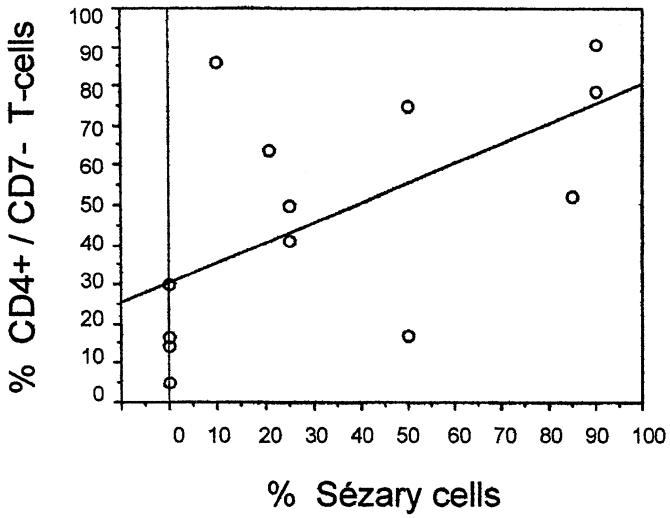

Figure 4. Percentage of Sézary cells counted in blood smears in correlation to the percentage of $CD4^+/CD7^-$ cells figured in a straight line.

lymphocytes and only 1–2% γδ T lymphocytes. The TCR-γ chain is the most suitable for clonality detection assays, especially by PCR, as its genome has a simple organization in comparison with the other TCR. Independent of the TCR expression, the γ locus is more frequently rearranged than the β locus, which remains in germline configuration in many cases (Theodorou *et al*, 1994). Why rearrangements of the "inappropriate" lineage are often found in mature T cells can be understood reviewing the TCR development during T cell ontogeny and αβ *versus* γδ lineage commitment in the thymus. MacDonald and Wilson (1998) suggest a modified separate lineage model that assumes a commitment to either the αβ or the γδ lineage prior to the TCR gene rearrangement. TCR development and signaling are important for further maturation and the main role of the αβ or γδ TCR is to rescue appropriate lineage-committed cells. The TCR β, γ, and δ genes rearrange in the same fashion in committed precursor cells of both lineages and they can undergo all possible rearrangements. Productively rearranged γδ genes are therefore also found in αβ T cells and vice versa; nevertheless, the majority of rearrangements of the "inappropriate" receptor genes are out-of-frame and non-productive (Kang *et al*, 1998).

Using the PCR approach we found demonstrable blood involvement in 35% of the patients in stage I and II. Similar data were reported earlier (Muche *et al*, 1997). We do not believe that this finding has prognostic implications, as the clinical course in these patients did not differ from PCR-negative cases.

Concerning the sequences of the rearrangements that were detected in stage III–IVb patients (SS patients and advanced CTCL), three of 13 patients presented a JγP1 or JγP2 segment use and failed detection with the standard primer panel (primer Vγ1–8, 9; Jγ1/2). In the 20 patients with CTCL in stage Ia–IIb, 18 of 20 patients (90%) presented clonality identified with the standard primer panel in skin biopsies.

Recently summarized data (Greiner, 1999) reveal the V and J segment usage of Vγ1–8 in 80% and Jγ1/2 in 60% of all T cells. Therefore, 60–80% of TCR rearrangements can be detected by our standard approach. Vγ9,10 and 11 are used in decreasing order. The frequency of JγP1/2 is estimated to be 7-11% (Födinger *et al*, 1996; Lynas and Howe, 1998). Leber *et al* (1989) suggested an early thymocytic origin of a lymphoma if a JγP1 segment is involved in the rearrangement, based on the observations of Quertermous *et al*, 1987 that JγP1 and JγP2 rearrangements are more frequently observed in thymocytes than in peripheral blood lymphocytes of healthy individuals. Other authors suggested that Vγ and Jγ rearrangement is developmentally ordered and that there is no

random usage of the segments (Goldman *et al*, 1993; Kohsaka *et al*, 1993; Bain *et al*, 1999). Although the number of cases investigated is rather low, we hypothesize that unproductive TCR rearrangements might be accompanied by an aggressive clinical behavior of malignant T cells.

Besides detection of circulating clonal cells, determination of the $CD4^+/CD7^-$ ratio in the peripheral blood is a marker for extracutaneous disease. The subset of $CD4^+/CD7^-$ T lymphocytes is expanded in cutaneous lesions of CTCL patients (Wood *et al*, 1990; Bergman *et al*, 1998). Therefore, it is proposed, that an increase of these cells in the peripheral blood is a sign for extracutaneous spread. The absolute number of $CD4^+/CD7^-$ T cells underestimates the frequency of the clonal cells, because we have recently shown that clonal cells are also present in $CD4^+/CD7^+$ T cell population in many CTCL patients (Dummer *et al*, 1999). Our findings demonstrate a correlation between $CD4^+/CD7^-$ frequency with higher stage and detection of clonal cells in the peripheral blood by PCR. Furthermore, a correlation of the $CD4^+/CD7^-$ percentage to the percentage of Sézary cells counted in blood smears from SS patients is noted, at least if there is no cut-off level (10% Sézary cells). This study demonstrates that both, molecular biologic analysis and determination of cell surface antigens are suitable markers for blood involvement. The combination of both is recommended for staging and monitoring of CTCL, because the prediction of peripheral blood involvement is more precise.

REFERENCES

Bain G, Romanow WJ, Albers K, Havran WL, Murre C: Positive and Negative Regulation of V (D) J Recombination by the E2A Proteins. *J Exp Med* 189:289–300, 1999

van Bakels V, Oostveen JW, Gordijn RLJ, Walboomers JMM, Meijer CJLM, Willemze R: Frequency and prognostic significance of clonal T-cell receptor β-gene rearrangements in the peripheral blood of patients with mycosis fungoides. *Arch Dermatol* 128:1602–1607, 1992

Bergman R, Faclieru D, Sahar D, *et al*: Immunophenotyping and T-cell receptor γ gene rearrangement analysis as an adjunct to the histopathologic diagnosis of mycosis fungoides. *J Am Acad Dermatol* 39:554–559, 1998

Bogen SA, Pelley D, Charif M, *et al*: Immunophenotypic identification of Sézary cells in peripheral blood. *Am J Clin Pathol* 106:739–748, 1996

Bourguin A, Tung R, Galili N, Sklar J: Rapid, nonradioactive detection of clonal T-cell receptor gene rearrangements in lymphoid neoplasms. *Proc Natl Acad Sci USA* 87:8536–8540, 1990

Bunn PA Jr, Lamberg SI: Report of the Committee on Staging and Classification of Cutaneous T-cell Lymphomas. *Cancer Treat Rep* 63:725–728, 1997

Bunn PA, Huberman MS, Whang-Peng J, *et al*: Prospective staging evaluation of patients with cutaneous T-cell lymphomas; demonstration of a high frequency of extracutaneous dissemination. *Ann Intern Med* 93:223–230, 1980

Burg G, Dummer R, Kerl H: Classification of cutaneous lymphomas. *Dermatol Clinics* 12:213–217, 1994

Burg G, Kempf W, Haeffner AC, *et al*: Cutaneous lymphomas. *Curr Prob Dermatol* 9:137–204, 1997

Dommann SNW, Dommann-Scherrer CC, Dours-Zimmermann MT, Zimmermann DR, Kural-Serbes B, Burg G: Clonal disease in extracutaneous compartments in cutaneous T-cell lymphomas. A comparative study between cutaneous T-cell lymphomas and pseudo lymphomas. *Arch Dermatol Res* 288:163–167, 1996

Dummer R, Häffner AC, Hess M, Burg G: A rational approach to the therapy of cutaneous T-cell lymphomas (CTCL). *Onkologie* 19:226–230, 1996a

Dummer R, Heald PW, Nestle FO, Ludwig E, Laine E, Hemmi S, Burg G: Sézary syndrome T-cell clones display T-helper 2 cytokines and express the accessory factor-1 (interferon-γ receptor β chain). *Blood* 88:1383–1389, 1996b

Dummer R, Nestle FO, Niederer E, *et al*: Genotypic, phenotypic and functional analysis of CD4+CD7+ and CD4+$CD7^-$ T lymphocyte subsets in Sézary syndrome. *Arch Dermatol Res* 291:307–311, 1999

Födinger M, Buchmayer H, Schwarzinger I, *et al*: Multiplex PCR for rapid detection of T-cell receptor-gamma chain gene rearrangements in patients with lymphoproliferative diseases. *Br J Haematol* 94:136–139, 1996

Goldman JP, Spencer DM, Raulet DH: Ordered rearrangement of variable region genes of the T cell receptor γ locus correlates with transcription of the unrearranged genes. *J Exp Med* 177:729–739, 1993

Greiner TC: Advances in molecular hematopathology: T-cell receptor γ and bcl-2 genes. *Am J Pathol* 154:7–9, 1999

Greiner TC, Raffeld M, Lutz C, Dick F, Jaffe ES: Analysis of T cell receptor-γ gene rearrangements by denaturing gradient gel electrophoresis of GC-clamped polymerase chain reaction products. *Am J Pathol* 146:46–55, 1995

Harmon CB, Witzig TE, Katzmann JA, Pittelkow MR: Detection of circulating T cells with CD4+$CD7^-$ immunophenotype in patients with benign and malignant lymphoproliferative dermatoses. *J Am Acad Dermatol* 35:404–410, 1996

Jakob T, Neuber K, Altenhoff J, Kowalzick L, Ring J: Stage-dependent expression of CD7, CD45RO, CD45RA and CD25 on CD4-positive peripheral blood T-lymphocytes in cutaneous T-cell lymphoma. *Acta Derm Venereol* 76:34–36, 1996

Kang J, Coles M, Cado D, Raulet DH: The developmental fate of T cells is critically influenced by TCRγδ expression. *Immunity* 8:427–438, 1998

Kohsaka H, Chen PP, Taniguchi A, Ollier WER, Carson DA: Regulation of the mature human T cell receptor γ repertoire by biased V-J gene rearrangement. *J Clin Invest* 91:171–178, 1993

Kukel S, Reinhold U, Oltermann I, Kreysel HW: Progressive increase of $CD7^-$ T cells in human blood lymphocytes with ageing. *Clin Exp Immunol* 98:163–168, 1994

Leber BF, Amlot P, Hoffbrand AV, Norton JD: Rearrangement of TCR gamma chain gene involving JP1 suggests early thymocyte origin of peripheral T-cell lymphoma. *Eur J Haematol* 42:368–374, 1989

Lutzner MA, Jordan HW: The ultrastructure of an abnormal cell in Sézary's syndrome. *Blood* 31:719–726, 1968

Lynas C, Howe D: Simple, reliable detection of T cell clones by PCR-LIS-SSCP analysis of TCRγ rearrangement. *Mol Cell Probes* 12:41–48, 1998

MacDonald HR, Wilson A: The role of the T-cell receptor (TCR) in αβ/γδ lineage commitment: clues from intracellular TCR staining. *Immunol Rev* 165:87–94, 1998

Meyer JC, Hassam S, Dummer R, Muletta S, Döbbeling U, Dommann SN, Burg G: A realistic approach to the sensitivity of PCR-DGGE and its application as a sensitive tool for the detection of clonality in cutaneous T-cell proliferations. *Exp Dermatol* 6:122–127, 1997

Muche M, Lukowsky A, Asadullah K, Gellrich S, Sterry W: Demonstration of frequent occurrence of clonal T cells in the peripheral blood of patients with primary cutaneous t-cell lymphoma. *Blood* 90:1636–1642, 1997

Quertermous T, Van Strauss WM, Dongen JJM, Seidman JG: Human T cell γ chain joining regions and T-cell development. *J Immunol* 138:2687–2690, 1987

Reinhold U, Abken H: CD4+ $CD7^-$ T cells: a separate subpopulation of memory T cells? *J Clin Immunol* 17:265–271, 1997

Reinhold U, Abken H, Kukel S, Moll M, Müller R, Oltermann I, Kreysel H: $CD7^-$ T cells represent a subset of normal human blood lymphocytes. *J Immunol* 150:2081–2089, 1993

Reinhold U, Liu L, Sesterhenn J, Abken H: $CD7^-$ negative T cells represent a separate differentiation pathway in a subset of post-thymic helper T cells. *Immunol* 89:391–396, 1996

Taylor JJ, Rowe D, Williamson IK, Christmas SE, Proctor SJ, Middleton PG: Detection of T-cell receptor γ chain V gene rearrangements using the polymerase chain reaction: application to the study of clonal disease cells in acute lymphoblastic leukemia. *Blood* 77:1989–1995, 1991

Theodorou I, Raphael M, Bigorgne C, *et al*: Recombination pattern of the TCR γ locus in human peripheral T-cell lymphomas. *J Pathol* 174:233–242, 1994

Weiss LM, Wood GS, Hu E, Abel EA, Hoppe RT, Sklar J: Detection of clonal T-cell receptor gene rearrangements in the peripheral blood of patients with mycosis fungoides/Sézary syndrome. *J Invest Dermatol* 92:601–604, 1989

Willemze R, Kerl H, Sterry W, *et al*: EORTC Classification for Primary Cutaneous Lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. *Blood* 90:354–371, 1997

Wood GS, Hong SR, Sasaki DT, Abel EA, Hoppe RT, Warnke RA, Morhenn VB: Leu/CD7 antigen expression by CD3+ T cells: comparative analysis of skin and blood in mycosis fungoides/Sézary syndrome relative to normal blood values. *J Am Acad Dermatol* 22:602–607, 1990

Wood GS, Tung RM, Haeffner AC, *et al*: Detection of clonal T-cell receptor γ gene rearrangements in early mycosis fungoides/Sézary syndrome by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). *J Invest Dermatol* 103:34–41, 1994