

# Detection of a Peripheral Blood T Cell Clone is an Independent Prognostic Marker in Mycosis Fungoides

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T cell receptor gene analysis is a sensitive method for assessment of peripheral blood involvement in mycosis fungoides. This study uses polymerase chain reaction/single-strand conformational polymorphism (PCR/SSCP) analysis of the T cell receptor  $\gamma$  gene and relates the results to skin stage and outcome in mycosis fungoides. Seventy-five peripheral blood samples from 66 patients were obtained from 1990 onwards and subjected to PCR/SSCP. Both Southern blot analysis and PCR/SSCP analysis were performed on 63 samples from 56 patients. Fourteen patients had T1 disease (12 IA, two IIA), 20 T2 (14 IB, five IIA, one IVA), 29 T3 (24 IIB, two IVA, three IVB, two patients tested at both T2 and T3), and five T4 (all III). The percentage of positive samples was higher with PCR/SSCP than with Southern blot

analysis (29 of 63 vs eight of 63 samples,  $p < 0.001$ ), and the percentage of positive samples increased with each stage (21% at T1, 35% at T2, 58% at T3, and 71% at T4). Proportional hazards analysis corrected for age, skin, and lymph node stage showed that the presence of a peripheral blood clone is associated with a worse outcome ( $p = 0.03$ , CI 1.1–6.03). These results indicate that the presence of a peripheral blood clone is an independent prognostic variable in patients with mycosis fungoides after correcting for age, skin, and lymph node stage, and that peripheral blood involvement is present in a large proportion of patients with early stage mycosis fungoides. **Keywords:** polymerase chain reaction/single-strand conformational polymorphism/T cell receptor gene rearrangement. *J Invest Dermatol* 114:117–121, 2000

**M**ycosis fungoides (MF) is the most common form of primary cutaneous T cell lymphoma. Four clinical stages of cutaneous disease are recognized and form the basis of the TNM classification: patches and plaques (T1 if  $< 10\%$ , T2 if  $> 10\%$  body surface area affected), tumors (T3) and erythroderma (T4) (Bunn and Lamburg, 1979). Skin stage has consistently been found to be the most important prognostic factor (Green *et al*, 1981; Marti *et al*, 1991). Systemic dissemination occurs more frequently with advanced cutaneous disease and is associated with a worse prognosis, but morphologic changes may be non-diagnostic in the early stages of lymph node and peripheral blood (PBL) involvement. Detection of a clonal T cell receptor (TCR) gene rearrangement using Southern blot analysis (SBA) is a more sensitive method of detecting the presence of a monoclonal T cell population in skin, blood, and lymph node (Weiss *et al*, 1985; 1989; Whittaker *et al*, 1991), and detection of a clonal population in lymph node specimens is associated with a poor prognosis (Lynch *et al*, 1992; Bakels *et al*, 1993; Kern *et al*, 1998). There are no published data, as yet, regarding the prognostic significance of more recently developed polymerase chain reaction (PCR) based methods of TCR gene analysis of the PBL in MF (Bourguin *et al*, 1990; McCarthy *et al*, 1991; Bottaro *et al*, 1994; Wood *et al*, 1994;

Muche *et al*, 1997). This study uses PCR/single-strand conformational polymorphism (SSCP) analysis of the TCR  $\gamma$  gene in PBL samples of patients with MF, compares results to those obtained using SBA, and relates these results to the clinical skin stage and outcome, to assess whether TCR gene analysis of PBL has any prognostic significance.

## MATERIALS AND METHODS

**Patients** Sixty-six patients with a clinical and histologic diagnosis of MF were included in a retrospective study. They had all been seen from 1990 onwards at St. John's Institute of Dermatology where PBL samples were obtained. The type of skin involvement (i.e., T1–4) at diagnosis, time of PBL sampling, and most recent follow-up were determined from the medical records. Other parameters recorded were the presence or absence of lymphadenopathy and results of radiologic investigations, full blood count, Sézary cell count, T cell subset analysis, and histologic findings of skin, lymph node, and bone marrow biopsies when performed. PBL samples from 14 patients with benign inflammatory dermatoses (five chronic actinic dermatitis, five pityriasis rubra pilaris, two eczema (including one erythrodermic), one erythrodermic psoriasis, one systemic lupus erythematosus) and five normal volunteer subjects were also included.

Fourteen patients had T1 disease (12 IA, two IIA), 20 T2 (14 IB, five IIA, one IVA), 29 T3 (24 IIB, two IVA, three IVB), and five T4 (all III). PBL samples were obtained from two patients at T2 and subsequently at T3. Twenty-two were female and 44 male.

**Specimens** Seventy-five blood specimens were obtained from 66 patients. Mononuclear cells were separated by sedimentation in gradients of Lymphoprep (Nycomed) and stored at  $-70^{\circ}\text{C}$  prior to DNA extraction using urea lysis or proteinase K digestion followed by phenol and chloroform. DNA was precipitated in ethanol, resuspended in Tris(hydroxymethyl)aminomethane-ethylenediamine tetraacetic acid

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Abbreviations: MF, mycosis fungoides; PBL, peripheral blood; SBA, Southern blot analysis; SSCP, single-strand conformational polymorphism.

(EDTA) and stored at  $-20^{\circ}\text{C}$ . Skin biopsy specimens were stored at  $-70^{\circ}\text{C}$  and homogenized prior to DNA extraction as above.

**SBA of TCR  $\beta$  gene** SBA was performed on EcoRI, HindIII, and in some cases BamHI digested genomic DNA using a cDNA probe for the C $\beta$  region of the TCR  $\beta$  gene as previously described (Whittaker et al, 1991).

**PCR analysis of TCR  $\gamma$  gene** Genomic DNA was subjected to PCR amplification on a Perkin Elmer GeneAmp PCR system 2400 (Perkin Elmer, Norwalk, CT) using different consensus primers for each of the four V $\gamma$  gene families and a consensus J $\gamma$  primer: V $\gamma$ I, TGCAGC-CAGTCAGAAATCTTCC; V $\gamma$ II, TGCAGGTCACCTAGAGCAACCT; V $\gamma$ III, AGCAGTTCCAGCTATCCATTTC; V $\gamma$ IV, TGCAAT-TGCACTTGGGCAGTTG; J $\gamma$ C, GACAACA/CAGTGTGTGTTCCAC. The reaction mix contained 200–500 ng genomic DNA, 250 ng of one of the four V $\gamma$  primers, 250 ng of the J $\gamma$  primer, 0.03 MBq  $^{32}\text{P}$  labeled dCTP, 0.2 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 0.1% Tween 20, 2 units Taq polymerase (Pharmacia), 10  $\times$  buffer (Pharmacia) and water to a final volume of 20  $\mu$ l. The reaction conditions were as follows: all primers, 5 min denaturation at  $94^{\circ}\text{C}$  prior to cycling and 5 min extension at  $72^{\circ}\text{C}$  after cycling; V $\gamma$ I, 30 cycles of 20 s at  $94^{\circ}\text{C}$ , 20 s at  $60^{\circ}\text{C}$  and 50 s at  $72^{\circ}\text{C}$ ; V $\gamma$ II, 30 cycles of 20 s at  $94^{\circ}\text{C}$ , 20 s at  $62^{\circ}\text{C}$  and 50 s at  $72^{\circ}\text{C}$ ; V $\gamma$ III and V $\gamma$ IV, 30 cycles of 20 s at  $94^{\circ}\text{C}$ , 20 s at  $58^{\circ}\text{C}$  and 50 s at  $72^{\circ}\text{C}$ .

A negative control containing all reagents except DNA was included in each PCR. DNA from a normal volunteer was used as a polyclonal control. DNA from the Jurkat T cell line (ECACC no. 88052401) was used as a monoclonal control for V $\gamma$ I and V $\gamma$ IV, and from patients with Sézary syndrome for V $\gamma$ II and V $\gamma$ III. 2.5  $\mu$ l aliquots were run on a 2% agarose minigel containing ethidium bromide and visualized under ultraviolet light.

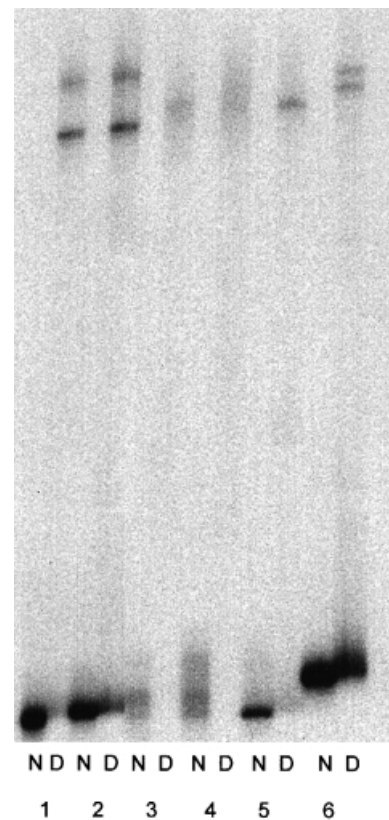
**SSCP analysis** According to the intensity of the band on the agarose gel 3–8  $\mu$ l aliquots of PCR product were diluted in 2  $\mu$ l of denaturing solution (0.1% sodium dodecylsulfate and 10 mM EDTA) and for each sample two 2  $\mu$ l aliquots of this mixture were diluted in an equal volume of formamide dye (formamide, xylene cyanol and bromophenol blue). Thus for each sample an aliquot of non-denatured and denatured mixture could be run. Denaturation was performed by heating to  $95^{\circ}\text{C}$  and snap-freezing on dry ice. The non-denatured and denatured samples were loaded in adjacent lanes on a 6% polyacrylamide gel containing 10% glycerol and subjected to electrophoresis using a V5 sequencing unit (Anachem, Luton, U.K.) at 10–12 mA overnight. The gel was then dried onto 3MM Whatman paper and subjected to autoradiography.

**Detection sensitivity** To determine the detection sensitivity of PCR/SSCP, DNA obtained from the Jurkat T cell line was serially diluted with DNA obtained from normal peripheral blood mononuclear cells and from the Daudi B cell line (ECACC no. 89120702). PCR/SSCP analysis using the V $\gamma$ I primer was then carried out as above.

**Sequencing** DNA eluted from the polyacrylamide gel was sequenced in five cases. After PCR/SSCP analysis as above, the area of the gel corresponding to a discrete band in the non-denatured or denatured sample was excised and the DNA was recovered by boiling the gel with water, incubation at  $-70^{\circ}\text{C}$  with glycogen, 3 M sodium acetate and ethanol, and centrifugation. Areas corresponding to smears were excised in two additional cases. The recovered DNA was reamplified using the appropriate V $\gamma$  primer and J $\gamma$ C primer. The PCR products were cleaned using a PCR product presequencing kit (Amersham Life Science, Cleveland, OH) according to the manufacturer's instructions, and the products were directly sequenced using a Thermosequenase radiolabeled terminator cycle sequencing kit (Amersham Life Science), according to the manufacturer's instructions.

## RESULTS

**PCR/SSCP analysis of the TCR  $\gamma$  gene (Fig 1)** Using PCR/SSCP analysis of the TCR  $\gamma$  gene, two patterns were seen. Smears could be seen in both the non-denatured sample and the denatured sample, reflecting a polyclonal pattern of V $\gamma$  gene usage. Alternatively, discrete bands could be seen in the denatured samples and, often with greater resolution, in the non-denatured samples, indicating the presence of a PCR product sequence amplified from a clonal TCR V $\gamma$  gene rearrangement. There was no PCR amplification of DNA extracted from the Daudi B cell line, which does not rearrange any TCR genes. No discrete bands



**Figure 1. PCR/SSCP analysis of the TCR V $\gamma$ I gene family.** PCR amplification was performed in this case using the V $\gamma$ I and J $\gamma$ C primers. N, non-denatured samples; D, denatured samples. Lanes 1 and 2, blood and skin, respectively, from a patient with T3 MF; lanes 3 and 4, different patients with plaque stage MF; lane 5, a patient with Sézary syndrome (incidental to this study); lane 6, Jurkat T cell line.

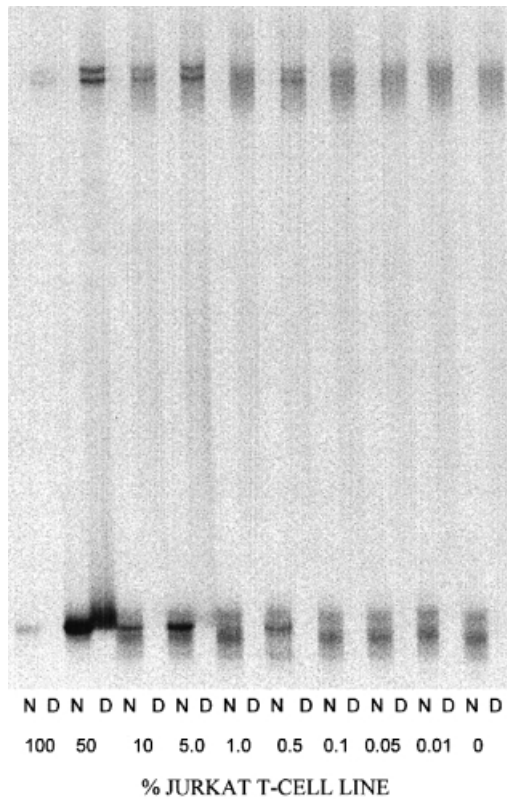
were seen in the samples from normal volunteers or patients with benign inflammatory dermatoses.

The detection sensitivity of this technique is approximately 0.5%; discrete bands were seen in the nondenatured and denatured samples containing 0.5% Jurkat DNA diluted with DNA extracted from a PBL sample from a normal volunteer (Fig 2).

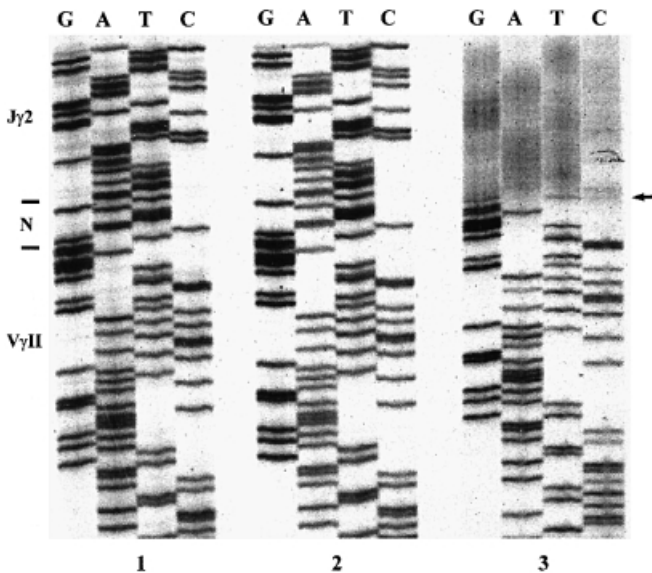
Sequence analysis was performed in five cases, and an identical TCR  $\gamma$  gene nucleotide sequence was obtained from the double-stranded and single-stranded bands in each case, indicating that the single-stranded bands represent conformers with either the same or complementary nucleotide sequence but different conformation (Fig 3). Sequencing of PCR products eluted from a smear using a V $\gamma$ II primer resulted in a clear V $\gamma$ II sequence which became unreadable close to the splice site where the N region sequence and J $\gamma$  sequence commence. This is consistent with the sequence that would be obtained from a polyclonal population. Sequence analysis of a case showing an identical band in blood and skin showed the same clonal sequence in both samples.

Overall, 30 of 66 patients had a clonal population detected using PCR/SSCP. Twelve patients showed rearrangement of the V $\gamma$ I gene (one patient also had rearrangement of V $\gamma$ II and another of V $\gamma$ III presumably reflecting biallelic  $\gamma$  TCR gene rearrangements), eight had rearrangement of V $\gamma$ II (one also had rearrangement of V $\gamma$ III), six had rearrangement of V $\gamma$ III (one with additional rearrangement of V $\gamma$ IV), and four had rearrangement of V $\gamma$ IV.

**PCR/SSCP is more sensitive than SBA and the percentage of positive samples increases with skin stage and overall clinical stage** Both SBA and PCR/SSCP analysis were performed in 63 PBL samples from 56 patients. The percentage of positive samples was higher with PCR/SSCP than SBA for each stage of skin disease. In total eight of 63 samples were positive with

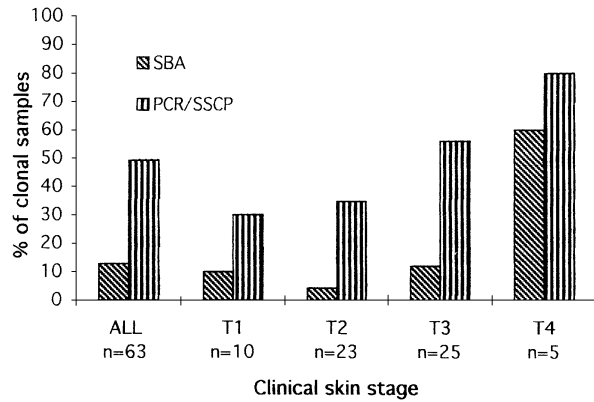


**Figure 2. Detection sensitivity of PCR/SSCP analysis of the TCR Vγ1 gene family.** DNA from Jurkat T cell line was serially diluted with DNA from a PBL mononuclear sample from a normal volunteer. N, nondenatured samples; D, denatured sample.



**Figure 3. Sequence analysis of DNA eluted from polyacrylamide gels using the VγII primer.** →, Splice site. Samples 1 and 2, analysis of DNA eluted from a band in the non-denatured and denatured lanes, respectively, from the same patient. The sequence shows the VγII and Jγ2 sequence separated by the N region. Sample 3, analysis of DNA eluted from a smear in the denatured lane.

SBA of the TCR β gene compared with 29 of 63 with PCR/SSCP analysis ( $p < 0.001$ , 95% confidence intervals for binomial distribution) (Fig 4). All patients who had a clone detected using SBA also had a detectable clone using PCR/SSCP analysis.



**Figure 4. Results of SBA and PCR/SSCP analysis on 63 samples.**

The percentage of positive samples increased with the stage of cutaneous disease: T1 21% ( $n = 14$ ), T2 35% ( $n = 23$ ), T3 58% ( $n = 31$ ), and T4 71% ( $n = 7$ ). Thus a significant proportion of patients with early cutaneous disease (i.e., patches and plaques) had evidence of a PBL T cell clone. Eight patients had repeat samples: one patient who was initially negative became positive without changing skin stage (T2) and two further patients became positive after progressing from T2 to T3 disease; the remaining five patients had identical results at each analysis without changing skin stage (two at T2, two at T3, and one at T4).

The percentage of patients with detectable circulating clones also broadly increased with overall clinical stage (IA,  $n = 12$ , 17%; IB,  $n = 14$ , 29%; IIA,  $n = 7$ , 71%; IIB,  $n = 22$ , 55%; III,  $n = 5$ , 60%; IVA,  $n = 3$ , 33%; IVB,  $n = 3$ , 100%). Of the seven patients with stage IIA, five did not have a lymph node biopsy and it is therefore possible that they in fact had stage IVA disease. Also, numbers in each group are small, and these factors may explain why the percentage at IIA is higher than at more advanced stages.

**Detection of a PBL clone is associated with a worse prognosis** Outcome data were obtained for 48 patients (24 T1 and T2, 24 T3) and correlated with the results of TCR gene analysis using proportional hazards analysis (Stata Statistical Software, Release 5.0, 1997; Stata, College Station, TX). Survival analysis was performed from the date of diagnosis, although TCR gene analysis was often performed only after a diagnosis of MF had been made. Four patients who died of causes other than MF were treated as censored observations. Robust standard errors were used to allow for clustering and non-normality, and which also adjust for the use of a normal approximation to a non-normal likelihood function (Lin and Wei, 1989). Using PCR/SSCP analysis to detect clonal disease, a hazard ratio of 2.6 was obtained ( $p = 0.034$ , CI 1.1–6.03) after adjusting for age, skin, and lymph node stage, thus indicating that PCR/SSCP analysis provides independent prognostic information on multivariate analysis. SBA was predictive of survival on univariate analysis but not on multivariate analysis, as too few cases were positive for it to be a useful predictor of outcome. Other significant prognostic indicators on multivariate analysis were skin stage T3 ( $p = 0.001$ ), and lymph node stages N1 ( $p = 0.012$ ) and N3 ( $p < 0.0001$ ).

The 10 y survival in patients with T1 and T2 disease was 50% in those without a detectable PBL clone and 30% in those with a PBL clone. The 5 y survival in both groups was 100%. In T3 disease, the median survival was 4.08 y in those patients without a clone and 2.42 y in those with a clone.

**Correlation of results of PCR/SSCP with lymphocyte morphology and T cell subset analysis** Small numbers of atypical lymphocytes were seen in three patients and a circulating T cell clone was detected in one of these cases. Sézary cells were seen in six further patients, but no patient had a Sézary count greater

than 5% PBL lymphocytes and therefore no patient fulfilled the criteria for Sézary syndrome. Of these patients, only two had a detectable circulating T cell clone.

T cell subset analysis was available for 31 patients; the CD4:CD8 ratio was elevated (normal less than 3.5) in only four cases. Two of these cases were clonal on SBA and PCR analysis (one at T1 and one at T3), whereas the other two cases were polyclonal on SBA and PCR analysis (one at T2 and one at T3).

## DISCUSSION

There are three aspects of this study which require further comment. First, we have compared the sensitivity of TCR gene analysis using PCR/SSCP analysis of the TCR  $\gamma$  gene with that of SBA of the TCR  $\beta$  gene in PBL samples from patients with MF, and have found PCR/SSCP to be significantly more sensitive than SBA. Secondly, we have shown that T cell clones in the PBL are detected more frequently with progressive stages of cutaneous disease and, finally, we found that detection of a PBL clone is an independent prognostic variable in MF.

Immunoglobulin and TCR gene analysis is now an established method for demonstrating the presence of monoclonal B or T lymphoid populations with identical clonal rearrangements of either the Ig or TCR genes, respectively. This was first performed using SBA (Arnold *et al*, 1983; Bertness *et al*, 1985), and more recently using techniques based on PCR amplification of the TCR or Ig genes and resolution of the PCR products by various different methods of electrophoresis (Bourguin *et al*, 1990; McCarthy *et al*, 1991; Davis *et al*, 1993; Volkenandt *et al*, 1993; Wood *et al*, 1994; Veelken *et al*, 1995). The ability to detect a clonal population is limited by the detection sensitivities of the different techniques: 1%–5% for SBA (Weiss *et al*, 1985), 0.5% for PCR/SSCP, and up to one in  $10^5$  with RNase protection assays (Veelken *et al*, 1995), but the latter method requires sequencing of individual TCR gene rearrangements and the generation of tumor-specific oligonucleotide probes and is therefore not suitable for routine analysis. TCR gene analysis has been performed on skin specimens from patients with MF using a variety of techniques (Bourguin *et al*, 1990; Whittaker *et al*, 1991; Volkenandt *et al*, 1993; Bottaro *et al*, 1994; Wood *et al*, 1994; Curco *et al*, 1997), with evidence of increased sensitivity of PCR-based methods compared to SBA (Wood *et al*, 1994; Curco *et al*, 1997). In lymph node specimens, SBA of the TCR genes has been used to demonstrate clonal populations in dermatopathic lymph nodes from patients with MF (Weiss *et al*, 1985), and that detection of a clone is associated with a poor prognosis (Lynch *et al*, 1992; Bakels *et al*, 1993; Kern *et al*, 1998). SBA of TCR genes is also a more sensitive method than morphologic examination for identification of patients with PBL T cell clones (Weiss *et al*, 1989; Whittaker *et al*, 1991). Again this has been related to prognosis: Bakels *et al* found that, of patients with lymph node involvement, those with a detectable PBL T cell clone had a much shorter median survival (Bakels *et al*, 1992). PCR/denaturing gradient gel electrophoresis analysis of the TCR  $\gamma$  gene on skin biopsy samples has been shown to predict response to treatment for MF (Delfau-Larue *et al*, 1998), but our study relates the results of PCR-based analysis of the PBL to prognosis.

PCR-based analysis has several advantages compared to SBA. It is rapid to perform, smaller amounts of DNA are required, and DNA extracted from paraffin sections may be used. Although techniques avoiding the use of radioactivity are advantageous, a greater detection sensitivity is obtained with radioactive labeling of PCR products (Bourguin *et al*, 1990). Different electrophoretic techniques have been used to resolve the PCR products, and those which involve a degree of denaturation also result in improved resolution. We elected to use PCR/SSCP, which was originally developed to detect point mutations and DNA polymorphisms as the difference of one nucleotide causes a band shift on polyacrylamide gel electrophoresis (Orita *et al*, 1989). PCR/SSCP has been used to detect clonal rearrangements of the immunoglobulin heavy chain gene in B cell malignancies (Davis

*et al*, 1993) and of the TCR  $\gamma$  in Sézary syndrome.<sup>1</sup> Because the PCR products are denatured before being loaded onto a non-denaturing gel, this method also allows non-denatured and denatured samples to be run in adjacent lanes, thus providing two complementary methods of analysis on the same gel. Our results show that the detection sensitivity of PCR/SSCP (0.5%) is an order of magnitude greater than that of SBA.

We have compared the results of PCR/SSCP analysis with those obtained using SBA. There are 64 different V $\beta$  gene segments arranged on the basis of sequence homology into 26 gene families, compared with 12 V $\gamma$  gene segments arranged into four families in addition to two pseudogenes (Arden *et al*, 1995). PCR amplification of all possible V $\beta$  gene rearrangements requires a large number of primers, whereas analysis of the TCR  $\gamma$  gene requires only four V $\gamma$  gene family specific primers. Discrete rearranged bands have previously been found using SBA of the  $\gamma$  TCR gene in all samples in which  $\beta$  TCR gene rearrangements are detected (Weiss *et al*, 1989; Whittaker *et al*, 1991).

The consensus primer for the J $\gamma$  region used amplifies rearrangements involving J $\gamma$ 1 and J $\gamma$ 2 genes (LeFranc *et al*, 1986a), but has 12–14 base pair mismatches for the three pseudogenes, J $\gamma$ P (LeFranc *et al*, 1986b), J $\gamma$ P1 (Huck and LeFranc, 1987), and J $\gamma$ P2 (Quertermous *et al*, 1987); it is therefore possible that rare, nonfunctional rearrangements involving these pseudogenes may not be amplified, giving false negative results. According to a study by Theodorou *et al*, the majority of V $\gamma$  gene rearrangements in human peripheral T cell lymphomas involve J $\gamma$ 1 or J $\gamma$ 2 (Theodorou *et al*, 1994). Their study did not include patients with MF, however, and further work is required to determine whether the frequency of J $\gamma$  gene segment usage is similar in MF and other peripheral T cell lymphomas.

MF is considered to be a primary cutaneous lymphoma and it is assumed that dissemination to blood, lymph nodes, and viscera occurs mainly with advanced disease; it is therefore not surprising that the proportion of patients with a detectable PBL clone is highest in patients with cutaneous tumors or erythroderma. We also detected PBL T cell clones in a significant proportion of patients with patch and plaque stage MF, confirming the findings of a recent study (Muche *et al*, 1997). These authors do not comment, however, about the prognostic significance of their findings, perhaps due to the fact that all patients with stage II–IV disease had detectable T cell clones in the PBL. The increased frequency of detection of PBL T cell clones in early stage MF reflects the increased sensitivity of PCR-based techniques which allow detection of a smaller tumor burden than is possible with SBA, and indicates that tumor cells are widely disseminated even in early cutaneous disease; in this respect our findings concur with those of Veelken *et al* (1995), where systemic involvement in patients with stage IA and IB MF was demonstrated using a highly sensitive technique (PCR/RNase protection analysis). Previous studies have detected identical T cell clones at different sites in patients with widespread cutaneous lesions and this is consistent with the concept that MF represents a neoplastic proliferation of memory T cells which preferentially home to skin whilst repeatedly circulating through PBL and local lymphatic tissue (Heald *et al*, 1993).

In previous studies of MF, the stage of skin disease has been found to be the most important prognostic variable (Green *et al*, 1981; Sauseville *et al*, 1988; Marti *et al*, 1991) in addition to advanced age (Weinstock and Horm, 1988) and the presence of visceral involvement (Sauseville *et al*, 1988). In erythrodermic MF, Kim *et al* found that overall clinical stage, age, and blood stage were independent prognostic variables (Kim *et al*, 1995). Other studies have shown that serum lactate dehydrogenase (Marti *et al*, 1991) and serum concentration of the soluble IL-2 receptor (Wasik *et al*, 1996), which both correlate with tumor burden, are important

<sup>1</sup>Hughes J, Ng Y, Spittle M, Smith N, Whittaker SJ. Comparison of Southern blot and SSCP-PCR analysis of TCR genes in the diagnosis of Sézary syndrome. *J Invest Dermatol* 105:457, 1995 (abstr.)

prognostic variables in cutaneous T cell lymphoma and erythrodermic cutaneous T cell lymphoma, respectively.

We have related the results of PCR/SSCP to outcome and have shown that the detection of a PBL clone is an independent prognostic variable after correcting for skin and lymph node stage, which almost certainly reflects the higher tumor burden in these patients. Patients with T3 stage disease and a detectable PBL clone have a shorter median survival than those T3 patients without a detectable PBL clone. The prolonged median survival in patients with patches and plaques will necessitate a longer period of follow-up, however, to determine whether there is also a difference in median survival in early stage disease between those with and those without a PBL clone. In early (T1 and T2) disease, our survival figures are better than those quoted by Marti *et al* (1991), but probably comparable to those of Green *et al* (1981) and Kim *et al* (1996, 1999). In patients with T3 stage disease, we found similar survival to that found in previous studies (Green *et al*, 1981; Marti *et al*, 1991). It will be interesting to compare the results of TCR gene analysis in patients with localized variants of MF such as pagetoid reticulosis as it is possible that such patients will not have evidence of disease dissemination.

To conclude, we report the use of PCR/SSCP of the TCR  $\gamma$  gene in MF. This is a novel technique for analysis of T cell malignancies and has been used in this study to demonstrate evidence of dissemination to PBL in patients with both early and advanced cutaneous involvement. By relating the results to outcome we have also shown that the results of PCR/SSCP are an independent prognostic variable in MF. These results require confirmation in larger, prospective studies, but suggest that PCR  $\gamma$  TCR gene analysis of PBL is an important additional staging method in MF.

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