Evidence that an Identical T Cell Clone in Skin and Peripheral Blood Lymphocytes is an Independent Prognostic Factor in Primary Cutaneous T Cell Lymphomas

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The monoclonality of the T cell receptor γ-chain gene was analyzed by polymerase chain reaction in skin and blood specimens of 85 patients with cutaneous T cell lymphomas including 67 mycosis fungoides, seven Sézary syndromes, and 11 CD30– nonepidermotropic cutaneous T cell lymphomas. A cutaneous T cell clone was detected in 69% of mycosis fungoides and 100% of Sézary syndromes. This frequency varied according to the clinical stage: 57% in early stages (Ia–IIa) to 96% in advanced stages (IIb–IV, Sézary syndrome). A peripheral blood T cell clone was detected in 42% of early stages and in 74% of late stages but was identical to the cutaneous one in 15% and in 63%, respectively. A significant association between initial clinical stage and T cell monoclonality was observed. In nonepidermotropic cutaneous T cell lymphomas, T cell monoclonality was detected in 55% of skin and 36% of blood samples. Univariate and multivariate analyses showed that, besides the initial clinical stage, an identical cutaneous and blood T cell clone was an independent prognostic factor for disease progression of mycosis fungoides/Sézary syndrome (hazard ratio 3.4, 95% confidence interval 1.4–9.9). Parallel polymerase chain reaction study of skin and blood specimens may therefore provide an initial prognostic marker that could help to monitor therapeutic strategies. A fully prospective study, with simultaneous therapeutic trials, needs to be done to confirm our findings and to include treatment variables in the statistical analysis. ***Key words: clonality/mycosis fungoides/cutaneous lymphomas. J Invest Dermatol 117:920–926, 2001***

Mycosis fungoides (MF) is the most frequent cutaneous T cell lymphoma (CTCL) and is characterized by a prolonged indolent evolution over several years or decades [5 y survival, 87% (Willemze *et al*, 1997)] and an epidermotropic infiltrate of cerebriform cells. Sézary syndrome (SS) consists in its leukemic counterpart with circulating cerebriform T cell lymphocytes (Sézary cells) associated with infiltrated erythroderma and generalized lymphadenopathy. Unlike MF, SS has a rapidly poor prognosis [5 y survival, 11% (Willemze *et al*, 1997)]. Adverse prognostic factors of MF/SS consist in age over 60 y (Diamandidou *et al*, 1999), skin involvement (T staging) (Marti *et al*, 1991; Zackheim *et al*, 1999), extracutaneous spread (Marti *et al*, 1991), presence of Sézary/atypical cells in the peripheral blood (Kim *et al*, 1995), and high lactate dehydrogenase levels (Marti *et al*, 1991; Diamandidou *et al*, 1999). Except for age, most of these factors are not predictive of MF/SS aggressiveness at initial diagnosis, but are correlated with the advanced stage of the disease. The group of nonepidermotropic CTCL is heterogeneous and subdivided into several entities according to the size of the lymphomatous cells and to the expression of CD30 antigen (Willemze *et al*, 1997). Although the group of CD30– nonepidermotropic CTCL is still incompletely defined (Willemze *et al*, 1997), the CD30+ nonepidermotropic CTCL is a separate entity of good prognosis with a 95% 5 y survival and different biologic behavior such as spontaneous regression (Paulli *et al*, 1995; Willemze *et al*, 1997; Vergier *et al*, 1998).

The diagnosis of CTCL relies on clinicopathologic characteristics but may be difficult especially at early stages. The detection of a dominant T cell clone in skin biopsy has been found as an additional argument for the diagnosis of CTCL (Weiss *et al*, 1985; Zelickson *et al*, 1991). Indeed, polymerase chain reaction (PCR) allowed the detection of monoclonal rearrangement of the T cell receptor γ chain (TCR-γ) gene in 53%–90% of MF/SS (Wood *et al*, 1994; Theodorou *et al*, 1995; Muche *et al*, 1997; Delfau-Larue *et al*, 1998a). The detection of cutaneous T cell monoclonality is more frequently achieved in advanced stages with infiltrated or tumoral lesions (73%–100%) than in MF with patch/plaque type lesions (50%–75%) (Bachelez *et al*, 1995; Curco *et al*,

Manuscript received December 13, 2000; revised May 13, 2001; accepted for publication May 23, 2001.

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Abbreviations: CR, complete remission; DGGE, denaturing gradient gel electrophoresis; MF, mycosis fungoides; PBL, peripheral blood lymphocytes; PR, partial remission; SS, Sézary syndrome.

1997; Muche *et al*, 1997; Delfau-Larue *et al*, 1998a). Moreover, the absence of a detectable cutaneous T cell clone is associated with a higher rate of complete remission (CR) (Delfau-Larue *et al*, 1998a). The detection of a T cell clone in peripheral blood lymphocytes (PBL) identical to the cutaneous clone has suggested that MF, even in the early stages, may be a systemic disease originating in the skin (Bakels *et al*, 1992; Muche *et al*, 1997; Delfau-Larue *et al*, 2000). The prognostic meaning of a circulating T cell clone has not been investigated in the above series of MF/SS. As in MF, a cutaneous T cell clone has been detected in 79%–100% of nonepidermotropic pleomorphic CD30– CTCL (Theodorou *et al*, 1995; Muche *et al*, 1997) but no correlation with stage or prognosis has been drawn owing to the recent provisional individualization of such diseases (Willemze *et al*, 1997).

The purpose of our study was to determine the frequency of T cell monoclonality, as determined by PCR in skin and PBL, in a cohort of CD30– CTCL, according to clinical stage and clinicopathologic group. The results were studied by comparison with the outcome of the patients to determine whether the initial PCR detection of T cell clone in skin, PBL, or both is associated with a poorer prognosis as an independent factor and may therefore be relevant for therapeutic strategies.

MATERIALS AND METHODS

Patient selection and staging system Patients with primary CTCL referred to the two dermatologic departments (Haut-Lévêque and Saint André Hospital) of the University Hospital of Bordeaux were prospectively included in this study between 1994 and March 2000. Inclusion criteria consisted of (i) a PCR study on a cutaneous biopsy representative for the diagnosis of CTCL and performed before any systemic therapy and (ii) a simultaneous PCR study in the skin and blood.

The patients were included with informed consent according to the French Bioethical law as skin and blood analyses were routinely performed at initial diagnosis. No additional biologic sample was taken for the purpose of the study. Moreover, the design of the study was approved by the Clinical Research Delegation of our University Hospital in January 1994.

The diagnosis of CTCL was made according to the EORTC classification for primary cutaneous lymphoma (Willemze *et al*, 1997). Thus, the cases were separated into MF, SS, and CD30– pleomorphic CTCL, with either large and medium-sized or small and medium-sized cells, whereas CD30+ large CTCL (more than 75% of the tumoral cells expressing the CD30 antigen) were excluded from the study as they are a distinct clinicopathologic entity.

The initial clinical stage of the patients with MF/SS was determined using the TNM classification adapted for MF (Bunn and Lamberg, 1979). All the nonepidermotropic CTCL included in our study were primary CTCL, defined by the absence of extracutaneous disease at the time of diagnosis and within the first 6 mo after diagnosis (Willemze *et al*, 1997). They were classified as either localized cutaneous disease (solitary or several lesions clustered at the same skin site) or diffuse cutaneous disease.

TCR-γ gene rearrangement analysis TCR-γ gene rearrangement was studied by using a GC-clamp multiplex PCR denaturing gradient gel electrophoresis (DGGE) as previously described (Theodorou *et al*, 1995). This procedure was originally demonstrated to amplify all possible TCR-γ gene rearrangements, as the primers are consensus and specific for each Vγ or Jγ segment family (Theodorou *et al*, 1995). Using different T cell lines or tumor monoclonal DNA, we also checked by monoplex PCR followed by sequencing analysis that each Vγ or Jγ consensus primer allowed us to amplify a defined monoclonal allele. The value of this procedure has also been demonstrated for the diagnosis of patients with epidermotropic T cell lymphoma (Theodorou *et al*, 1995; Delfau-Larue *et al*, 1998a, b, 2000). In our hands, the sensitivity threshold for the detection of a dominant T cell clonal rearrangement ranged from 2.5% to 5% of clonal cells diluted into polyclonal cells depending on the rearranged allele. The sensitivity was checked throughout the study by including in each assay a dilution of DNA extracted from Jurkat cell line (kindly provided by P. Cornillet, Reims, France). PCR study was performed on DNA extracted from the frozen half of cutaneous biopsies processed for histopathologic examination and on mononuclear blood cells purified by density gradient centrifugation. Cutaneous biopsies were taken as the most infiltrated lesion in each patient and the presence of an epidermotropic CTCL infiltrate was checked on frozen sections before DNA extraction. Samples were interpreted either as polyclonal (presence of a smear) or as monoclonal (presence of one to four dominant or discrete bands depending on the formation of homoduplicities or heteroduplicities) (Theodorou *et al*, 1995). When a T cell clone was detected in the cutaneous specimen, it was considered as the reference clone for comparison with the T cell clone possibly detected in PBL. Indeed, the use of GC-clamp primers and DGGE in our PCR assay resulted in a genetic imprint specific for the TCR-γ allele that was useful for comparison of both the size and sequence of PCR products (Theodorou *et al*, 1995). This allowed us to classify the cases in three main groups: group 1, polyclonal profile in the skin whatever the profile in blood; group 2, T cell clone in skin without an identical clone in PBL (where PCR detected either a polyclonal profile or a different T cell clone); group 3, identical T cell clone in skin and PBL according to DGGE analysis.

Statistical analyses The prognostic value of clonality was assessed using survival analysis methods. The entry date of our study sample was the histologic diagnosis of MF. Patients were followed up every 6 mo. They were considered lost to follow-up if their last follow-up occurred more than 6 mo before September 2000. Disease progression was evaluated retrospectively by the two physicians who have followed all patients. The interval to disease progression was calculated from the entry date to the date of diagnosis of ''events'' [absence of response to treatment, progression of cutaneous lesions, or occurrence of extracutaneous involvement (both leading to change in the stage of the disease)] or to the date of death. Patients with complete or partial remission (CR/PR) until last clinical follow-up were right-censored. The study focused on the frequency and the prognostic value of initial T cell clone PCR detection and did not consider the different treatments received by the patients. Indeed, treatments were administered according to the initial clinical stage and could not therefore be evaluated as an independent variable as no therapeutic trial was conducted prospectively. The multivariate analysis of clonality effect was therefore adjusted on age, gender, and clinical stage. When clinical events other than death occurred between two visits, the observations were interval-censored. Therefore, univariate and multivariate analyses were performed using a proportional hazards model with a penalized likelihood approach (Joly *et al*, 1998), and comparison with a right-censored approach using SAS software, version 6.12 (Allison, 1995), showed no significant difference between these two approaches. The proportional hazards assumption was graphically checked by examining hazard functions.

RESULTS

Overall, 85 patients (60 men and 25 women) were included. The average age was 59 y (18–92 y). According to the clinicopathologic diagnosis based on EORTC classification (Willemze and Meijer, 2000), the patients were classified as 67 MF including eight transformed MF, seven SS, and 11 CD30– pleomorphic CTCL (either small/medium-sized or medium/large-sized cells: $n = 8$ and $n = 3$, respectively). The TNM classification (Bunn and Lamberg, 1979) allowed us to classify the 67 patients with MF as 18 stage Ia, 25 stage Ib, four stage IIa, eight stage IIb, six stage IIIa, three stage IIIb, two stage IVa, one stage IVb. The seven SS were classified as six stage IVa and one stage IIIb. Stages Ia–IIa were considered as early stages and stages IIb–IVb (including SS) as advanced stages. The 11 CD30– nonepidermotropic CTCL were classified as localized ($n = 5$) and diffuse ($n = 6$) skin disease.

T cell clone PCR detection frequency according to clinicopathologic group and initial stage of disease According to the clinicopathologic group, cutaneous T cell monoclonality frequency varied as follows: 69% for MF (46 of 67), 100% for SS (seven of seven), and 55% for CD30– CTCL (six of 11).

Mycosis fungoides and Sézary syndrome (**Fig 1**) Regardless of the initial stage of the disease, 72% (53 out of 74) of the patients with MF/SS had a cutaneous T cell clone and 54% (40 of 74) had a blood T cell clone. The T cell clone PCR detection frequency varied according to the clinical stage, from the early stages (Ia–IIa: 57% in the skin and 42% in PBL) to the advanced stages (IIb–IV SS: 96% in the skin and 74% in PBL). When detected, the T cell clone in PBL was significantly more frequently identical to the cutaneous

reference clone in advanced than in early stages (17 of 20 *vs* 7 of 20, $p = 0.001$). Then, 15% of early MF had an identical clone in skin and PBL *versus* 63% for advanced MF.

According to the clonality results (**Table I**), the patients were classified as: group 1, 42% of early stages, 4% of advanced stages; group 2, 43% of early stages, 33% of advanced stages; group 3, 15% of early stages, 63% of advanced stages.

Statistical analysis demonstrated that clinical stage (early/advanced stages) and clonality were highly associated (Pearson $\chi^2 = 21.7$, $p < 10^{-4}$).

CD30–Nonepidermotropic CTCL Whatever the stage, 55% (six of 11) of patients showed T cell monoclonality in skin and 36% (four of 11) in PBL. The clonality results seemed to vary between patients with localized or diffuse cutaneous disease as, respectively, none out of five and three out of six had an identical T cell clone in skin and blood. Moreover, four of the five cases with localized cutaneous disease had a polyclonal profile in the skin. Then, five cases were in group 1 (four localized and one diffuse cutaneous disease), three in group 2 (one localized and two diffuse cutaneous disease), and three in group 3 (all had diffuse cutaneous disease). Lastly, the results of clonality were not found to be associated with the histologic subtype (three cases with pleomorphic medium/large-sized cells: two in group 1 and one in group 3).

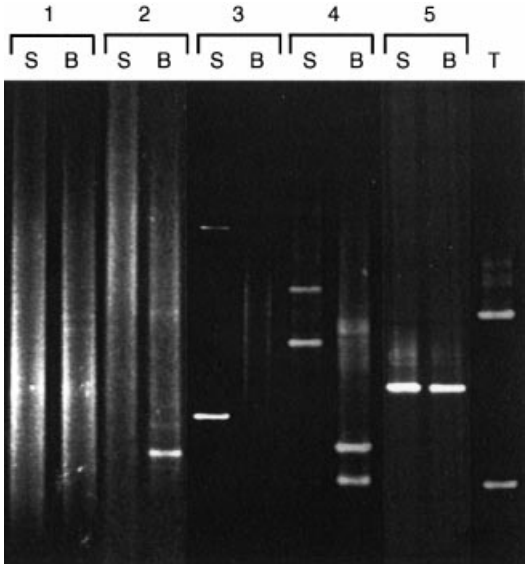

Figure 1. Amplification of the TCR-γ gene rearrangement. PCR products were run on a DGGE polyacrylamide gel and were ethidium bromide stained. S, skin; B, blood; T, positive control (Jurkat cell line). Group 1 (cases 1 and 2): A polyclonal profile was observed in skin with either a polyclonal profile (case 1) or a T cell clone (case 2) in blood. Group 2 (cases 3 and 4): A monoclonal rearrangement of the TCR-γ gene in skin with either a polyclonal profile (case 3) or a different T cell clone (case 4) in blood. Group 3 (case 5): An identical T cell clone was observed in skin and blood.

Outcome according to the PCR results The median follow-up time in the study was 32 mo (ranging from 5 to 77 mo) and was comparable between the different clinical stages (**Table II**). Death occurred in 10 patients including seven MF, two SS, and one CD30– CTCL, and as it was a rare event, survival was not a good criterion to evaluate the prognostic value of PCR detection in CTCL. Therefore, the prognostic evaluation was performed as described above: CR/PR as opposed to absence of response to treatment or progression. Stable disease (absence of response to treatment) was retained as an ''event'', grouped with ''progression''. Indeed, all the patients at early stages with a stable disease ($n = 4$) finally progressed or died after 8–25 mo, whereas those at late stages with a stable disease progressed ($n = 6$) or did not respond to treatment ($n = 4$). By definition, the patients included did not receive systemic treatment before the PCR study.

Treatments were administered according to clinical stage and were relatively homogeneous within each stage group for most of the patients, but no prospective therapeutic trial was simultaneously performed. At early stages (Ia–IIa), most of the patients were treated by topical therapies (corticosteroids, topical nitrogen mustard, carmustine, psoralen with ultraviolet A). At advanced stages, radiotherapy was performed on tumors (IIb, T3), interferon, chlorambucil, methotrexate, or polychemotherapy were used for stages IIIa–IVb, and extracorporeal photopheresis or chemotherapy for SS.

The group of MF/SS included 74 assessable patients. Two MF Ib, group 1 were lost to follow-up.

First, we compared the status of the patients at the end of the study. Among the 19 patients in group 1, 17 (89%) achieved CR/PR. In the 29 patients in group 2, 19 (65%) experienced CR/PR. In the 24 patients in group 3, CR/PR was obtained in only six (25%).

Factors associated with disease progression were studied with special emphasis on clonality results (**Table III**, **Fig 2**). Neither age nor sex was associated with disease progression. Conversely, clinical stage (early *versus* advanced) was an important prognostic factor, as the risk of disease progression was higher in advanced compared to early stages in univariate [hazard ratio (HR) 6.7, $p < 10^{-4}$] and multivariate (HR = 5, $p = 8 \times 10^{-4}$) analyses. The clonality results were also found to be a significant variable for the risk of progression in univariate ($p < 10^{-4}$) as in multivariate ($p = 0.02$)

Table I. PCR study in MF/SS according to initial clinical stage ($N = 74$)

	Group 1 No T cell clone in skin n (%)	Group 2 T cell clone in skin only n (%)	Group 3 Identical T cell clone in skin and blood n (%)	Total n (%)
Ia	8^{1}	7^{3}	3	18
Ib	11^{5}	10^{3}	4	25
IIa	1	3^{1}	0	4
Early stages	**20^{6} (42)**	**20^{7} (43)**	**7 (15)**	**47 (100)**
IIb	0	4^{2}	4	8
IIIa	0	2	4	6
IIIb	1	1	1	3
IVa	0	1	1	2
IVb	0	1^{1}	0	1
SS	0	0	7	7
Advanced stages	**1 (4)**	**9^{3} (33)**	**17 (63)**	**27 (100)**

[a]Numbers in exponent: number of cases with clonal but not identical rearrangement in PBL

analysis. On comparison of groups 2 and 3 (clonality in skin whatever the results in PBL) with group 1 (cutaneous polyclonality) we observed a significantly higher risk of progression in univariate analysis (HR = 3.6, p = 0.02) but not in multivariate analysis. When a T cell clone was detected only in the skin, it was not statistically associated with disease progression. On the other hand, when the cutaneous clone was associated with an identical T cell clone in blood (group 3), it was associated with a poorer prognosis, compared with an isolated cutaneous T cell clone (group 2) (HR = 3.5, 95% confidence interval 1.5–8.2). Thus, multivariate analysis demonstrated that an identical T cell clone in PBL and skin consisted in an independent risk factor for disease progression in MF/SS.

Nonepidermotropic CD30– CTCL were analyzed separately as their clinical behavior and prognosis are different from MF/SS. Moreover, the size of this group did not allow adequate statistical analysis.

Among the five patients in group 1 (polyclonal cutaneous profile), the four patients with localized cutaneous disease were in CR/PR whereas the patient with diffuse cutaneous disease progressed. All the patients in group 2 (isolated cutaneous T cell clone), including one localized and two diffuse cutaneous disease, were in CR/PR. Lastly, only one of the three patients (diffuse cutaneous disease) in group 3 (identical clone in skin and blood) was in CR whereas the other two had progressed (n = 1) or were deceased (n = 1).

Table II. Median follow-up according to the clinical stage in MF/SS (N = 74)

Stage	Number of cases	Median follow-up (range)	
Ia	18	47.5	(29–77 mo)
Ib	25	29	(5–60 mo)
IIa	4	27.5	(24–40 mo)
IIb	8	15.5	(6–61 mo)
IIIa	6	24	(11–37 mo)
IIIb	3	45	(24–57 mo)
IV	3	54	(6–67 mo)
SS	7	30	(6–65 mo)
Total	74	32	(5–77 mo)

DISCUSSION

In this series, patients with MF/SS and CD30– nonepidermotropic CTCL referred to our departments were evaluated with no bias of

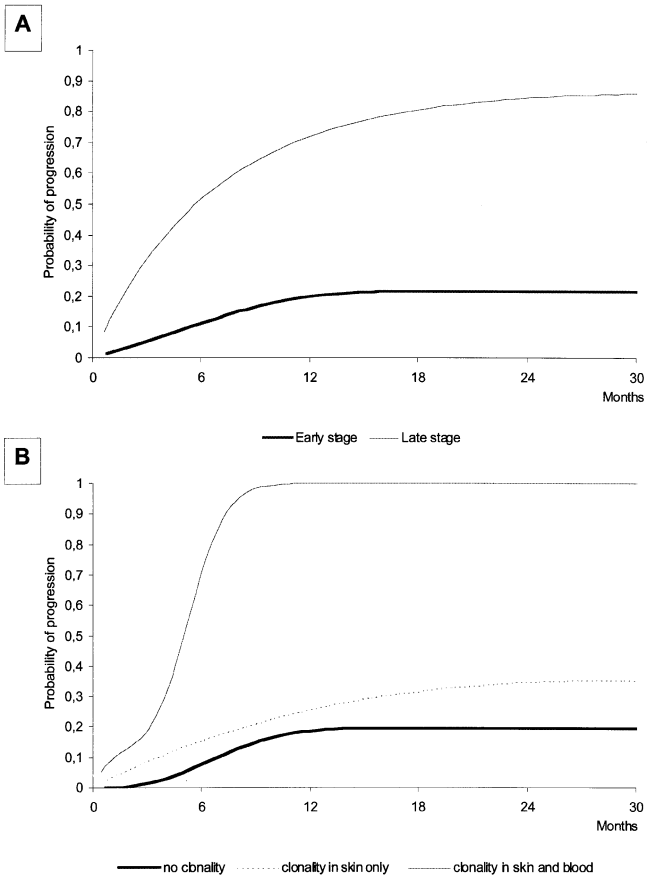

Figure 2. Statistical analysis. (*A*) Probability of progression of the patients with MF/SS according to the initial clinical stage (early stage *versus* late stage). (*B*) Probability of progression of the patients with MF/SS according to the clonality results in skin and blood (no T cell clone in skin, T cell clone in skin only, identical T cell clone in skin and blood).

Table III. Prognostic factors of disease progression in MF/SS (N = 74)[a]

	Description		Univariate analysis[b]		Multivariate analysis[b]		
Variable	N	No. of events (%)	HR	p	HR	95% CI	p
Sex:							
Female	23	10 (43)	1.0		1.0		0.92
Male	51	22 (43)	1.1	0.74	0.96	0.44–2.1	
Age:							
⩽ 60 y	37	12 (32)	1.0		1.0		0.78
> 60 y	37	20 (54)	1.9	0.08	1.1	0.51–2.4	
Clinical stage:							
Early	47	10 (21)	1.0	$< 10^{-4}$	1.0	2.0–12.7	8.10^{-4}
Advanced	27	22 (81)	6.7	$< 10^{-4}$	5.0		0.02
Clonality:							
No T cell clone in skin	21	4 (19)	0.52		1.02	0.28–3.64	
T cell clone in skin only	29	10 (34)	1.0[c]		1.0[c]		
Identical T cell clone in skin and blood	24	18 (75)	3.9		3.4	1.4–9.9	

[a]HR, hazard ratio; CI, confidence interval.
[b]Proportional hazards model.
[c]Reference.

inclusion depending on the clinical presentation or histologic subtype. The frequency of T cell monoclonality in skin and blood specimens was determined at initial diagnosis time and its prognostic value was retrospectively analyzed according to follow-up every 6 mo of each patient by the two physicians who followed all patients. Skin or blood T cell monoclonality was found to parallel the initial stage of the disease prior to therapy. The presence of an identical T cell clone in skin and blood was demonstrated by multivariate analysis as an independent factor for disease progression.

Frequency of T cell clone according to stage and clinicopathologic group Molecular genetic techniques have demonstrated the clonal nature of cutaneous lymphomatous cells in CTCL. This was first revealed in the tumoral stage of MF and in SS by southern blot analysis of TCR-β gene rearrangement (Weiss *et al*, 1985; Ralfkiaer *et al*, 1987). This technique allowed the detection of T cell monoclonality in most (> 90%) advanced stages (erythroderma, tumoral lesions) but infrequently at early stages with noninfiltrated patch lesions (Weiss *et al*, 1989; Whittaker *et al*, 1991; Zelickson *et al*, 1991; Therune *et al*, 1993). Using PCR, a monoclonal TCR-γ gene rearrangement was detected in most cutaneous specimens of MF/SS (50%–100% according to the stage) (Wood *et al*, 1994; Theodorou *et al*, 1995; Muche *et al*, 1997; Delfau-Larue *et al*, 1998a, 2000). The overall 69% T cell monoclonality in our MF/SS cases is an intermediate value between the frequency of 53% reported by Theodorou *et al* (1995), who studied mostly patients with patches and plaques, and that of 90% reported by Wood *et al* (1994), who studied patients at advanced stages. Delfau-Larue *et al* (1998a), who detected a cutaneous T cell clone in 62% of patients with MF in variable stages (mostly early stages), suggested that T cell monoclonality may be directly related to the density of lymphomatous cells in the skin infiltrate but did not find a correlation with cutaneous or extracutaneous extension. Muche *et al* (1997) found a cutaneous T cell monoclonality in 70%–75% of MF/SS whatever the stage. Our series of a representative sample of MF/SS patients with an average age of 58 y is therefore the first that statistically demonstrates a significant relationship between clonality and clinical stage.

Using PCR, several groups have shown the presence of monoclonal circulating T cells in SS and at advanced stages of MF, although less frequently at the early stages such as MF Ia (Wood *et al*, 1994; Theodorou *et al*, 1995; Veelken *et al*, 1995; Curco *et al*, 1997; Muche *et al*, 1997; Delfau-Larue *et al*, 2000; Fraser-Andrews *et al*, 2000). Our study also showed that blood T cell monoclonality was correlated with initial clinical stage prior to therapy in unselected patients with CTCL (54% of all cases: 42% in early stages and 74% in advanced stages). The PCR-DGGE technique allowed us to compare the electrophoretic profile of blood T cell clonal rearrangement with the cutaneous one. This revealed that identical monoclonal T cells are detectable before therapy both in skin and blood more frequently at advanced stages (63%) than in patients at early stages (15%) of MF/SS. Our data are in accordance with the rates of blood T cell monoclonality (45%) reported by Fraser-Andrews *et al* (2000) in MF/SS, but their blood specimens were not studied in comparison with cutaneous ones, except for one case studied with comparative sequence analysis, which is a laborious procedure for routine analysis. Similar to our PCR-DGGE, Muche *et al* (1997) used heteroduplex-loaded temperature gradient gel electrophoresis without systematic sequencing and obtained a rate of detection of blood T cell clonality ranging from 46% in early stages (MF Ia) to 100% in advanced stages (MF II–IV). Recently, Delfau-Larue *et al* (2000), also using the same multiplex PCR-DGGE technique, reported that rates of blood clonality were variable according to the clinical stage (4%–68% according to the stage).

Moreover, we detected monoclonal blood T cells different to the cutaneous clone, especially at early MF stages (28% *vs* 11% in late stages). Muche *et al* (1997) have previously reported such unrelated PBL T cell clones in two of 58 specimens in a parallel PCR study of skin and blood. Delfau-Larue *et al* (2000) have recently shown that unrelated PBL T cell clones may indeed be detected in up to 33% of patients with MF or with benign cutaneous infiltrates. Moreover, these unrelated PBL T cell clones were more frequently observed in patients over 60 y of age, in accordance with the detection of monoclonal T cells in PBL of normal elderly donors over 65 y of age (Posnett *et al*, 1994). In our study, the mean age of the 34 patients without a blood T cell clone (53.5 y) did not significantly ($p = 0.15$) differ from that of the 16 patients with an unrelated blood T cell clone (68.5 y). An unrelated PBL T cell clone appeared more frequently in patients with early stage MF (28%) than in those with advanced disease (11%), suggesting that it may correspond to an activated CD8+ clonal population as a host immune response to the tumoral clone (Asadullah *et al*, 1997; Muche *et al*, 1999). Immunophenotyping of blood cells, especially at early stages of MF, might be useful to determine whether clonal T cells belong to CD4+ or CD8+ lymphocytes and to analyze their T cell receptor repertoire.

Interestingly, all cases of SS had an identical T cell clone in skin and blood whereas the number of circulating Sézary cells was below 10% in three out of seven patients (our personal results). Blood clonality has been proposed as a major diagnostic criterion in SS (Willemze *et al*, 1997; Fraser-Andrews *et al*, 1998) rather than the subjective morphologic identification of Sézary cells (Weinberg *et al*, 1995). Moreover, monoclonal T cells may be detectable in blood but not in skin specimens of patients with SS (Muche *et al*, 1997; Willemze *et al*, 1997). Such a situation was not observed in our series.

Very few data are available for nonepidermotropic CTCL, with a detection frequency of TCR-γ rearrangement that varies from 50% to 100% in the skin (Theodorou *et al*, 1995; Dommann *et al*, 1996; Muche *et al*, 1997) and from 50% to 75% in PBL (Theodorou *et al*, 1995; Muche *et al*, 1997). These studies lacked information about histologic subtype, however, including CD30 phenotype and stage of lymphoma. The 55% rate of cutaneous T cell monoclonality in nonepidermotropic CD30– CTCL is in accordance with the above data. The presence of an identical blood T cell clone in three out of 11 patients without extracutaneous disease suggests an early blood circulation of tumoral cells, as in MF. The frequency of T cell clone in skin and blood also appeared to be related to the extent of the disease (localized *versus* diffuse cutaneous disease). Such results underline the similarities between MF and nonepidermotropic CD30– CTCL, especially for the pleomorphic small/medium-sized subtype (eight out of 11 patients), which is a provisional entity in the EORTC classification (Willemze *et al*, 1997).

An identical cutaneous and blood T cell clone is an independent prognostic factor in MF/SS Whereas several groups have shown T cell monoclonality of cutaneous or blood specimens in MF/SS, only two groups have recently demonstrated its significant prognostic value (Delfau-Larue *et al*, 1998a; Fraser-Andrews *et al*, 2000). The first study showed that the detection of T cell monoclonality in cutaneous lesions of early MF is predictive of the achievement of CR (Delfau-Larue *et al*, 1998a). CR is a highly variable notion according to time-point follow-up and definition of CR duration, however. Moreover, CR was not found to be correlated with a better survival in MF (Kaye *et al*, 1989; Kim *et al*, 1999). Therefore, CR may not necessarily be the major therapeutic goal, which is rather to improve either survival or the quality of life by obtaining a regression of cutaneous lesions. The second study showed that the presence of a blood T cell clone was an independent prognostic marker in MF according to the 10 y survival (50% in patients without a detectable blood clone *versus* 30% in those with a PBL clone), whereas the 5 y survival was not statistically different, as in our study (Fraser-Andrews *et al*, 2000). For the above reasons, we found it more reliable to assess the prognostic value of the PCR study in terms of disease progression rather than CR or survival. By studying T cell clonality both in skin and blood specimens at the time of diagnosis, we found two main

prognostic factors for MF/SS: (i) the initial stage of the disease was confirmed as an independent prognostic factor (Diamandidou *et al*, 1999; Zackheim *et al*, 1999); (ii) the presence of an identical clone in skin and blood. First, we showed that the presence of a cutaneous T cell clone whatever the results in PBL had a significant unfavorable value only by univariate analysis. When the prognostic value of an isolated cutaneous T cell and an identical T clone in skin and blood was successively analyzed, we demonstrated by multivariate analysis that only the presence of an identical T cell clone in skin and blood is an independent prognostic factor. The cytologic detection of peripheral blood abnormal T cells is a marker of poor prognosis in MF/SS but such detection is not a reproducible or sensitive assay (Schechter *et al*, 1987; Kim *et al*, 1995). In our study, the detection of a blood T cell clone had a prognostic value even for early MF stages and in the absence of cytologically detectable abnormal peripheral blood cells. As only identical blood and skin T cell monoclonality had a prognostic value, our study points to the need for using PCR analysis such as DGGE allowing a direct comparison of the TCR-γ rearranged allele in routine analysis (Theodorou *et al*, 1995). The sensitivity threshold of around 5% for our technique allowed prognostic study although the prognostic significance of a lower threshold (sensitivity of 0.1%), such as in the study of Muche *et al* (1997), remains to be evaluated. The emergence of real-time quantitative PCR will also allow standardized clonality analyses to be obtained, which should be useful for prognostic studies (Verhagen *et al*, 2000).

In the absence of identified recurrent molecular abnormality in MF/SS, TCR-γ gene rearrangement analysis is so far the unique molecular analytical technique for managing these lymphomas, as a parallel PCR study of skin and blood specimens appears in our study of clinical interest at diagnosis for evaluating the risk of progression. The subdivision of patients according to initial molecular staging would help to evaluate therapeutic strategies. Therefore, the detection of circulating tumoral lymphocytes in early stages of MF would allow evaluation of the interest of systemic immunologic rather than local therapies before the disease is clinically widespread. A fully prospective study, with simultaneous therapeutic trials, needs to be done to confirm our findings and to include treatment variables in the statistical analysis. Moreover, longitudinal studies would determine whether a molecular follow-up will define patients with a higher risk of progression as the clonal TCR gene rearrangement usually remains unchanged over time (Bottaro *et al*, 1994; Theodorou *et al*, 1995; Delfau-Larue *et al*, 1998b).

We thank C. Bartoli and J. Ferrer for their expert technical assistance. This work was supported by a grant from the Programme Hospitalier de Recherche Clinique (DRC Aquitaine).

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