T-cell receptor γ gene rearrangement in cutaneous T-cell lymphoma: comparative study of polymerase chain reaction with denaturing gradient gel electrophoresis and GeneScan analysis

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Summary

The usefulness of T-cell receptor gene rearrangement (TCR-GR) analyses for differentiating cutaneous T-cell lymphoma (CTCL) from benign inflammatory disorders (BID) has been insufficiently studied to date.

Objectives To evaluate the diagnostic value of TCR-GR analyses, comparing polymerase chain reaction (PCR) with denaturing gradient gel electrophoresis (DGGE) analysis and BIOMED-2 standardized protocol PCR with GeneScan analysis (BIOMED-2-GS).

Methods Both types of PCR were performed in 157 patients evaluated for initial features suggestive of CTCL between 1996 and 2007. After clinical and histological review, the final diagnosis was CTCL in 77 cases and BID in 80 cases.

Results DGGE and BIOMED-2-GS had a similar diagnostic value for distinguishing CTCL from BID, with a sensitivity of 74% and 77%, respectively, and a specificity of 86%. The observed concordance between both methods was 90% and the kappa coefficient was 0.79. Positivity rates did not depend on the PCR method but varied according to the type of CTCL (73–75% in mycosis fungoides, 90–100% in Sézary syndrome, 40–60% in lymphomatoid papulosis and 100% in other types). The positivity rate in BID was 14% with both methods. The most frequent BID with a monoclonal pattern were drug-induced cutaneous lymphoid hyperplasia, erythrodermic psoriasis and pityriasis lichenoides chronica.

Conclusions BIOMED-2-GS analysis of the TCRγ gene is as sensitive and specific as DGGE for CTCL diagnosis. In addition, BIOMED-2-GS is less time-consuming and gives more information concerning the size and nature of TCR-GR.

The diagnosis of primary cutaneous T-cell lymphoma (CTCL) classically relies on a combination of clinical, pathological and immunophenotypical criteria. However, the distinction between CTCL and benign lymphoproliferative or inflammatory disorders (BID) may still be difficult using these criteria, particularly in early stages of CTCL and in some cases of erythroderma or reactive lymphoproliferations.

Although monoclonality as a single criterion cannot be considered synonymous with malignancy, recent studies found the assessment of clonality by different molecular techniques to be helpful in distinguishing benign from malignant cutaneous lymphocytic infiltrates.1–3 In addition, the detection of a clonal T-cell receptor gene rearrangement (TCR-GR) in blood and lymph nodes has been introduced as a staging procedure in the new tumour, node, metastasis (TNM) classification of mycosis fungoides (MF) and Sézary syndrome.4 However, the actual diagnostic value of TCR-GR analysis has not been sufficiently evaluated to date.

Previous studies were heterogeneous and often limited to small numbers and/or by selection biases of specific diagnoses, which made results poorly applicable in real-life practice to a large spectrum of diseases. In addition, numerous polymerase chain reaction (PCR) protocols were used with different primers or amplification methods; the data were thus not comparable. Techniques mostly used for the detection of TCR-GR included TCRβ or TCRγ gene analysis either by Southern blot or by PCR, using polyacrylamide gel electrophoresis, denaturing gradient gel electrophoresis (DGGE) or
heteroduplex analysis. More recently, the TCRγ-GR was studied by PCR and GeneScan (GS), using fluorescently labelled PCR primers and capillary electrophoresis. Few studies to date have been performed using GS, most of which included fewer than 50 cases. To the best of our knowledge, only two studies (limited to 32 and 35 patients, respectively) compared GS with DGGE.5 6 Finally, only rare studies have evaluated the specificity of TCR-GR detection for CTCL diagnosis using a large control series of various reactive diseases.1 7

This prompted us to analyse retrospectively, in our real-life practice of more than 10 years, the diagnostic value of TCRγ-GR analysis in CTCL and various BID, and to compare the results obtained using PCR analysis with DGGE and the BIOMED-2 standardized protocol PCR with GS analysis (BIOMED-2-GS).

Materials and methods

Inclusion and review process

Since 1996, a TCR-GR analysis using DGGE has been routinely performed at initial evaluation of patients with CTCL in the Department of Dermatology of Reims University Hospital, France. From January 1996 to January 2007, 200 patients had a cutaneous biopsy taken for TCR-GR analysis, either for a CTCL, or for other (most often benign) diseases in which CTCL had initially been considered as a possible diagnosis. Among these 200 patients, 184 met the following inclusion criteria. (i) Available clinical data from medical records, including accurate description of initial cutaneous lesions and/or clinical photographs, initial staging and informative follow-up. (ii) Available material for histological review. (iii) Cryopreserved skin sample obtained from a representative lesion, including sufficient DNA material for additional molecular analysis using BIOMED-2-GS.

These 184 cases were studied according to a standardized procedure. First, histology was reviewed by two dermatopathologists (A.D. and A.L.G.), blinded to clinical data. Slides stained with haematoxylin, eosin and safranin were studied in all cases. CD3, CD4, CD8, CD20, CD30 and/or CD68 stainings were performed, as usually indicated for diagnosis. At the end of the pathological review, all cases were histologically classified as CTCL, BID, or uncertain cases.

Secondly, a final diagnosis (Table 1) was established by two dermatopathologists (F.G. and A.L.G.) and two dermatopathologists (A.D. and A.L.G.) blinded to molecular analysis, using the histopathological review, clinical data including initial evaluation and follow-up, and informative clinical pictures. Patients with CTCL were classified according to the World Health Organization–European Organization for Research and Treatment of Cancer (EORTC) classification,8 and to the modified TNM staging system of the International Society for Cutaneous Lymphomas and the Cutaneous Lymphoma Task Force of the EORTC.4 9

Finally, cryopreserved specimens were blinded and evaluated for TCR-GR without knowledge of the final diagnosis. In all cases, the DGGE analysis was reviewed, and samples were analysed using BIOMED-2-GS.

T-cell receptor gene rearrangement analysis

Genomic DNA was prepared from 1 mm3 of frozen cutaneous biopsy using DNeasy tissue kit® (Qiagen, Courteboeuf, France) according to the manufacturer’s instructions.

Polymerase chain reaction and denaturing gradient gel electrophoresis

To amplify the TCRγ locus, a multiplex PCR using four GC-clamped primers for the V segments (respectively, consensus for Vγ1–8, Vγ9, Vγ10 and Vγ11) and three primers for the J segments (respectively, consensus for Jγ1.1 and Jγ1.2 (Jγ1.1/Jγ1.2), Jγ2.1 (Jγ2.1), and Jγ1.3 and Jγ2.3 (Jγ1.1/Jγ2.1)) was performed in a single tube, followed by DGGE, as previously reported.10 Clonal T-cell populations were determined by visual inspection of ethidium bromide-stained gels and categorized as follows: monoclonal if one to four prominent bands were found, or polyclonal if no distinct band per smear could be observed.

Table 1 Detection of T-cell receptor (TCR) gene rearrangement by polymerase chain reaction (PCR) analysis with denaturing gradient gel electrophoresis (DGGE) and BIOMED-2 standardized protocol PCR with GeneScan analysis (BIOMED-2-GS) in 157 cases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
</tr>
<tr>
<td>CTCL</td>
<td>77</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>52</td>
</tr>
<tr>
<td>Stage Ia</td>
<td>21</td>
</tr>
<tr>
<td>Stage Ib</td>
<td>12</td>
</tr>
<tr>
<td>Stage IIa</td>
<td>4</td>
</tr>
<tr>
<td>Stage IIb</td>
<td>9</td>
</tr>
<tr>
<td>Stage III</td>
<td>4</td>
</tr>
<tr>
<td>Stage IV</td>
<td>2</td>
</tr>
<tr>
<td>Early stage (I–IIa)</td>
<td>37</td>
</tr>
<tr>
<td>Late stage (IIb–IV)</td>
<td>15</td>
</tr>
<tr>
<td>Sézary syndrome</td>
<td>10</td>
</tr>
<tr>
<td>Lymphomatoid papulosis</td>
<td>10</td>
</tr>
<tr>
<td>CTCL, other</td>
<td>5</td>
</tr>
<tr>
<td>BID</td>
<td>80</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>11</td>
</tr>
<tr>
<td>Eczema</td>
<td>27</td>
</tr>
<tr>
<td>CLH</td>
<td>11</td>
</tr>
<tr>
<td>Drug-induced CLH</td>
<td>8</td>
</tr>
<tr>
<td>Idiopathic CLH</td>
<td>3</td>
</tr>
<tr>
<td>Prurigo</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>28</td>
</tr>
</tbody>
</table>

CTCL, cutaneous T-cell lymphoma; BID, benign inflammatory disorders; CLH, cutaneous lymphoid hyperplasia.
BIOMED-2 standardized protocol polymerase chain reaction and fluorescent capillary electrophoresis analysed by GeneScan software

For fragment analysis, a multiplex PCR using four primers for the V segments and two labelled primers for the J segments were divided into two tubes: PCR A, Vγ1–8 + Vγ10 + FAM-labelled Jγ1.1/1.2 + VIC-labelled Jγ1.3/2.3 primers; and PCR B, Vγ9 + Vγ11 + FAM-labelled Jγ1.1/1.2 + VIC-labelled Jγ1.3/2.3 primers.

Primers were chosen according to the European BIOMED-2 PCR collaborative study instructions. The size of the PCR products was determined using the GeneScan 3.1 computer software (Applied Biosystems, Foster City, CA, U.S.A.). In all cases two determinations were systematically performed, as previously recommended. In the light of both analyses, the final BIOMED-2-GS result was determined as follows (Fig. 1).

The following cases were considered as monoclonal. (i) Cases with a dominant T-cell clone, when one or two distinct fluorescent peaks at least twice as intense as the background, and with appropriate size ranges were reproducible (in size and/or intensity) in both PCR assays. (ii) Cases defined as oligoclonal which had more than two peaks on first determination, and one or two major peaks reproducible at second determination, whereas additional peaks were not.

The following cases were considered as polyclonal. (i) Cases with reproducible typical Gaussian curves. (ii) Cases with non-reproducible electrophoresis patterns. This included cases with a monoclonal peak found on only one analysis and cases with two different peaks on duplicate analyses. This was considered as pseudomonoclonality. (iii) Ambiguous cases after the two GS analyses: these cases had reproducible electrophoresis peaks which were weak in intensity and associated with an important polyclonal background. In addition, the type of rearrangement family in these cases corresponded to canonical or usual reactive rearrangement.

Control samples

The quality of primers was controlled on four patients known for the type of TCRγ rearrangement. Sensitivity of the PCR methods was evaluated with dilution of the Jurkat T-cell line DNA (1% and 10%) in DNA from normal leucocytes. The detection threshold was reproducible at 10% for DGGE and at 10% and 1% for BIOMED-2-GS. The specificity of PCR methods was controlled using two polyclonal isolated leucocyte samples. For BIOMED-2-GS evaluation, 20 reactive skin biopsies were used as control samples to identify canonical and physiologically overexpressed rearrangements. These samples were obtained from 10 patients with initial suspicion of bullous pemphigoid who had a negative direct immunofluorescence (inflammatory skin taken for immunofluorescence analysis) and 10 patients with melanoma (normal skin specimens taken for complementary excision).

Statistical analysis

Data were summarized using frequencies, percentages, means and ranges. χ² tests, Student’s t-tests and Fisher’s exact tests were used for comparisons of means and percentages according to numbers, as appropriate. A difference was considered statistically significant when P < 0.05. The kappa coefficient of Cohen was used to evaluate correlation between DGGE and BIOMED-2-GS, and was calculated as: \( \kappa = (P_o - P_e)/(1 - P_e) \), Po being the observed agreement percentage and Pe the random agreement percentage. To evaluate the diagnostic value of DGGE and BIOMED-2-GS, CTCL was chosen as the disease to be diagnosed and BID were considered as controls; the detection of monoclonality was considered as a positive test and polyclonality as a negative result. The common definitions of sensitivity, specificity, positive predictive value and negative predictive value were then used.

Results

Patients

Among 184 cases selected for the review process, 27 cases were excluded because the final diagnosis at the end of the review process either remained uncertain (19 cases) or was that of systemic lymphoma with secondary cutaneous involvement (eight cases). The remaining 157 patients were finally diagnosed with either CTCL (77 cases) or cutaneous BID (80 cases) and were extensively studied. This group included 99 males and 58 females with a mean age of 59 years (range 10–90). The mean follow-up from initial evaluation was 35.2 months (range 0–123) for CTCL and 19.5 months (range 0–143) for BID. The mean length of time from first symptoms to last follow-up was 75.7 months (range 3–334) and 53 months (range 2–316) in the two groups, respectively.

The distribution of patients according to diagnosis is shown in Table 1. Among those with CTCL, 52 (68%) had MF, 10 (13%) had Sézary syndrome and 10 (13%) had lymphomatoid papulosus. The remaining five patients had primary cutaneous anaplastic large-cell lymphoma (n = 1), primary cutaneous CD4+ small/medium-size pleomorphic T-cell lymphomas (n = 2), other primary cutaneous peripheral T-cell lymphoma (n = 1) and indolent CD8+ lymphoid proliferation of the ear (n = 1). The most frequent final diagnoses in 80 patients with BID were eczema (n = 27), psoriasis (n = 11), cutaneous lymphoid hyperplasia (CLH) (n = 11, including eight cases of drug-induced eruptions) and prurigo (n = 3). Less frequent diagnoses were lupus erythematosus, Jessner–Kanof disease, pityriasis lichenoides chronicus, acne, morphoea, pyoderma gangrenosum, pityriasis rubra pilaris, chronic actinic dermatitis, panniculitis, lichen planus and insect bite.

Rates of T-cell receptor gene rearrangement

The results of both BIOMED-2-GS analyses were concordant in 134 samples, including 49 monoclonal cases, 65 poly-
clonal cases, eight oligoclonal cases finally classified as monoclonal and 12 ambiguous cases finally classified as polyclonal. In 23 cases, discrepancies were observed between duplicate BIOMED-2-GS analyses. These cases were finally classified by comparison of both results, as monoclonal in 13 cases and polyclonal in 10 cases, using criteria defined above.

Rates of TCR-GR by diagnosis using both techniques are shown in Table 1. Overall, a monoclonal TCR-GR was found in 74% and 77% of cases of CTCL using DGGE and BIOMED-2-GS, respectively, and in 14% of cases of BID with both techniques. Sensitivities, specificities, positive and negative predictive values for the diagnosis of CTCL vs. BID were similar using DGGE and BIOMED-2-GS (Table 2). In CTCL,
the rate of TCR-GR depended on the type of lymphoma (Table 1): 90–100% in Sézary syndrome, 40–60% in lymphomatoid papulosis, about 75% in MF and 100% in other types of CTCL. We found no significant difference for TCR-GR between early and late stages of CTCL. However, using DGGE, rearrangements were slightly more frequent in late stages (87% vs. 70%, P = 0.2). Among BID with monoclonal pattern (14%), the most frequent were CLHs. Those CLHs found to be monoclonal with one or both techniques were drug-induced CLHs. Drugs responsible for these monoclonal drug-induced CLHs were allopurinol (n = 1), amitriptyline (n = 1), enoxaparin (n = 1) and indapamide (n = 1). Other monoclonal BID were cases of eczema, psoriasis, morphea, discoid lupus erythematosus and pityriasis lichenoides chronica.

Comparison between denaturing gradient gel electrophoresis and BIOMED-2 standardized protocol polymerase chain reaction with GeneScan analysis

Rates of TCR-GR (Table 1) observed in both CTCL and BID were similar using DGGE and BIOMED-2-GS. There was complete agreement between both techniques in 141 of 157 cases (90%) (Table 3). Patients with concordant results from both techniques totalled 61 positive cases and 80 negative cases. The kappa coefficient of Cohen was 0.79, indicating a good concordance.

Nine cases with a polyclonal result using DGGE produced clonal bands with BIOMED-2-GS, and seven monoclonal cases with DGGE were polyclonal with BIOMED-2-GS, leading to a disagreement rate of 16 of 157 cases (10%).

Table 3 Concordance between polymerase chain reaction (PCR) analysis with denaturing gradient gel electrophoresis (DGGE) and BIOMED-2 standardized protocol PCR with GeneScan analysis (BIOMED-2-GS) for T-cell receptor γ gene rearrangement analysis in 77 patients with cutaneous T-cell lymphoma (CTCL) and 80 patients with benign inflammatory disorders (BID)

<table>
<thead>
<tr>
<th></th>
<th>DGGE+</th>
<th>DGGE-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CTCL (n = 77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIOMED-2-GS+</td>
<td>53</td>
<td>6</td>
</tr>
<tr>
<td>BIOMED-2-GS-</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Patients with BID (n = 80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIOMED-2-GS+</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>BIOMED-2-GS-</td>
<td>3</td>
<td>66</td>
</tr>
</tbody>
</table>

Table 4 Type of rearranged Vγ segments in the monoclonal T-cell population identified with both polymerase chain reaction (PCR) analysis with denaturing gradient gel electrophoresis and BIOMED-2 standardized protocol PCR with GeneScan analysis in 53 cases of cutaneous T-cell lymphoma (CTCL) and eight cases of benign inflammatory disorders (BID)

<table>
<thead>
<tr>
<th>TCR-GR</th>
<th>Totala</th>
<th>V1–8</th>
<th>V9</th>
<th>V10</th>
<th>V11</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTCL</td>
<td>72 (100%)</td>
<td>57 (79%)</td>
<td>8 (11%)</td>
<td>7 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>BID</td>
<td>12 (100%)</td>
<td>6 (50%)</td>
<td>2 (17%)</td>
<td>4 (33%)</td>
<td>0</td>
</tr>
</tbody>
</table>

TCR-GR, T-cell receptor gene rearrangement. *Nineteen patients with CTCL and four patients with BID had double TCRγ gene rearrangements.

The types of TCR-GR in the 61 cases (i.e. 53 CTCL and eight BID, Table 3) in which monoclonality was detected using both methods are shown in Table 4. Among 84 Vγ segment rearrangements observed in these 61 patients, a different distribution was observed between both groups. In patients with CTCL, 79% of all rearrangements involved the Vγ8 family, as compared with 50% in patients with BID (P = 0.04). Rearrangements observed in BID and normal skin (data not shown) were frequently involved other families, i.e. the Vγ9, Vγ10 and Vγ11 families. In addition, clones in BID and normal skin frequently corresponded to prominent peaks observed on Gaussian curves of negative patients, for example: Vγ9–Jγ1.3/2.3 (178 bp), Vγ10–Jγ1.3/2.3 (160 bp), Vγ11–Jγ1.3/2.3 (95 bp) and Vγ8–Jγ1.3/2.3 (240 bp).

Discussion

In the present study, we investigated the diagnostic value of TCR-GR analysis in a large series of patients with CTCL and BID and compared DGGE with BIOMED-2-GS. To our knowledge, this is the largest study comparing these two methods. We observed a similar, high diagnostic value using both methods. Sensitivity was around 75% and specificity 86%. The observed concordance rate (90%) and the kappa coefficient value (0.79) were indicative of a good concordance of DGGE and BIOMED-2-GS. These results confirm and extend previous studies in smaller series and allow for further conclusions.

The frequency of monoclonality in CTCL was 74% with DGGE and 77% with BIOMED-2-GS (Table 1). Previous studies using DGGE or GS in CTCL reported rates of monoclonality ranging from 60% to 89.7%. However, few studies...
included more than 50 patients,\textsuperscript{1,7,10,17–20} were carried out on frozen specimens,\textsuperscript{1–3,6,7,10,16,18,21,22} used standardized PCR primers\textsuperscript{2,5} and analysed TCR\textsuperscript{\gamma}-GR.

Some studies analysed TCR\textsubscript{\beta}-GR,\textsuperscript{13,24} and reported higher rates of monoclonality in CTCL, but the technique is more difficult and more time-consuming than TCR\textsuperscript{\gamma}-GR analysis. The simpler organization of the TCR\textsuperscript{\gamma} locus and the fact that this locus undergoes early and preserved rearrangement makes it more suitable than the \textbeta locus for the determination of clonality by single-round PCR from genomic DNA-based methods.\textsuperscript{15} However, the limited heterogeneity of the TCR\textsuperscript{\gamma} requires high-resolution electrophoresis to separate PCR products by PCR methods such as DGGE, heteroduplex analysis or GS. Although GS allows an accurate comparative evaluation of the size and the V-family type of clonal TCR\textsuperscript{\gamma}-GR products, only a few studies have been performed to date using this technique.\textsuperscript{1,3,12,13,17,24,26–28} In 2003, a European BIOMED-2 collaborative study published a design and standardization of PCR primers and protocols for detection of clonal TCR\textsuperscript{\gamma}-GR in suspected malignant lymphoproliferation. To date, few studies have evaluated this protocol in cutaneous diseases.\textsuperscript{2,5}

Beside variations due to different techniques, the rate of monoclonality may vary greatly between studies according to the selection of patients and the type of lymphoma. In our series, all consecutive cases investigated for a possible CTCL in our real-life practice had a TCR-GR analysis. In addition, the relatively high number of cases allowed for analysis in different subgroups of patients according to the type and the stage of CTCL (Table 1). In previous studies using various techniques, rates of monoclonality in early CTCL ranged from 57\% to 71\%, and often appeared lower than in later stages.\textsuperscript{18,29–31} In the present study, the rate of monoclonality in early MF was around 70\% and did not differ from that of late stages, indicating a good diagnostic value of both BIOMED-2-GS and DGGE from early stages of MF. Our high rate of monoclonality observed in the skin of patients with Sézary syndrome (90\% with DGGE and 100\% with BIOMED-2-GS) was in agreement with the 78–100\% rates reported in previous studies\textsuperscript{21,32} in Sézary syndrome or erythodermic MF. The only patient with Sézary syndrome and a polyclonal cutaneous sample at first DGGE evaluation in our series had subsequent cutaneous biopsies during the disease progression, which displayed a monoclonal pattern with DGGE. This patient was found to be positive with BIOMED-2-GS at first analysis. In contrast, we detected a monoclonal TCR-GR in only 40\% (DGGE) to 60\% (BIOMED-2-GS) of patients with lymphomatoid papulosis, which was in accordance with the 22–70\% rates previously reported.\textsuperscript{10,27}

Fourteen patients with MF had no detectable monoclonal TCR-GR using either method. Overall, the rate of negative CTCL was 23\% and 26\% using BIOMED-2-GS and DGGE, respectively. This failure to detect clonal T-cell populations may have various causes. (i) A true absence of TCR\textsuperscript{\gamma} rearrangements is a usual characteristic of rare types of lymphomas, such as natural killer type or lymphoblastic lymphomas. However, such lymphomas were not present in our series. (ii) An incomplete or deleterious rearrangement within the TCR\textsuperscript{\gamma} locus can prevent the binding of PCR primers. (iii) Finally, but most probably in our series, clonal T cells may be below the detection threshold of the method, particularly in cases with a weak histological cutaneous lymphoid infiltrate.\textsuperscript{18,33}

Fourteen patients with BID had a monoclonal pattern either with DGGE or with BIOMED-2-GS. This included four cases of drug-induced CLH and two cases of pityriasis lichenoides chronica, both diseases which have previously been reported to be commonly associated with monoclonal TCR-GR.\textsuperscript{7,34,35} Other BID with a monoclonal pattern were three cases of eczema, three of psoriasis, one of morphoea and one of discoid lupus erythematosus. In previous studies, rates of monoclonality in BID ranged from 0\% to 31\%, depending on the PCR method used\textsuperscript{44} and the type of BID analysed. The rate of monoclonality in patients with psoriasis (three of 11, 27\%) is high in our study. However, it must be recalled that only patients whose clinical features initially led to a suspicion of CTCL were evaluated for TCR-GR. These three patients first presented with a severe erythroderma which required hospitalization. Chang et al.\textsuperscript{16} also reported a persistent monoclonal pattern in some patients with psoriasis. The hypothesis of a reactive clone activated by various antigens may be discussed. Further studies are warranted to evaluate whether some particular clinical and/or histological patterns may be frequently associated with monoclonality in psoriasis.

We observed a similar diagnostic value and a good concordance rate between BIOMED-2-GS and DGGE (Tables 2 and 3). Most previous studies which compared GS with other PCR methods found that GS had an equal or better sensitivity, mainly due to a better detection threshold (0–1–1\%, as compared with 1–10\% for other methods). Only one study\textsuperscript{7} reported a lower sensitivity of BIOMED-2-GS (58\%) as compared with heteroduplex PCR (65–4\%). Both previous studies\textsuperscript{5,6} which compared GS and DGGE in small numbers of patients found an equal sensitivity of both techniques.

Nine cases (6\%) were classified as monoclonal with BIOMED-2-GS analysis and polyclonal with DGGE. These cases were six patients with MF and three with BID. These results indicate that BIOMED-2-GS may show a better sensitivity than DGGE in some cases. This may result from the use of fluorescent labelled primers and optimized BIOMED-2 protocol\textsuperscript{11} in two separate tubes, which prevent competition between rearrangements present.

Seven cases (four MF and three BID) were positive with DGGE and negative with BIOMED-2-GS. A second analysis using both techniques confirmed these results. These results may be due to the presence of PCR products with sizes out of the detectable range of BIOMED-2-GS, or to a particular rearrangement of Vj recognized by DGGE primers and missed by BIOMED-2-GS primers. The JP primer was used in our DGGE analysis, but not in BIOMED-2-GS with BIOMED-2 protocol primers. This choice of the BIOMED-2 European consensus was justified by the fact that JP rearrangements are frequent canonical, nonspecific rearrangements and are very rare in CTCL.

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In the present study, two independent BIOMED-2-GS analyses were performed in all cases, as recommended. Ten cases (4%) with an ambiguous result after the first BIOMED-2-GS analysis were classified as negative in view of duplicate analyses. In these cases, PCR products analysed by BIOMED-2-GS showed in first analysis a monoclonal appearance that was not reproducible in sizes and/or in peak-height ratios in an independent PCR assay. This phenomenon, referred to as pseudomonoclonality, was first described by Dippel et al. It can originate from a very low DNA input or from small subordinate T-cell clones. Pseudomonoclonality may be explained by the cellular composition of the lesions when proliferating T cells are embedded in a background of numerous polyclonal lymphoid cells, leading to amplification of only a few or even a single TCR-GR of various sizes. In such cases, the TCR-GR does not appear reproducible when further independent GS analyses are performed.

Eight patients with an oligoclonal pattern (5%) were observed after the two BIOMED-2-GS analyses. All eight cases were finally considered positive, because the high intensity of peaks and the absence of polyclonal background were suggestive of a tumorous clone rather than a reactive lymphoid proliferation. Two of these eight cases were negative with DGGE and corresponded to lymphomatoid papulosis and lichenoid pityriasis, respectively. The other six were positive with DGGE and corresponded to CTCL (four early MF, one lymphomatoid papulosis, one primary cutaneous small pleomorphic T-cell lymphoma). This oligoclonal pattern has occasionally been reported in CTCL and reactive BID. Oligoclonality in CTCL may be explained by the proportion of infiltrative reactive T cells resulting from the anti-tumour response. This oligoclonal pattern was found to be associated predominantly with earlier tumour stages.

BIOMED-2-GS may have technical advantages compared with DGGE. The capillary electrophoresis used in BIOMED-2-GS requires neither harmful substances such as ethidium bromide and polyacrylamide, nor fragile DNA sequencing gel matrices, as with DGGE. In addition, it provides semiquantitative results which can be accurately analysed, as in some cases of the present study. Thus it appears to be a time-saving and technically simple method.

Furthermore, BIOMED-2-GS may be more accurate in some cases, especially in those with a polyclonal background or with monoclonal T cells representing <1% of the cutaneous infiltrate. The precise assessment of the length of PCR products allows for a comparison of T-cell clones from various samples of the same patient, without sequencing. The type of rearrangements can be defined by measuring the size of PCR products and the colour of fluorochrome. By measuring the peak heights, the method may allow an estimation of the relative amount of T-cell clones among the polyclonal backgrounds.

In summary, we found that DGGE and BIOMED-2-GS analysis of TCRγ PCR products are both sensitive and specific methods that may usefully contribute to the differential diagnosis of CTCL and reactive BID. Our data provide strong arguments in favour of the systematic use of TCR-GR analyses in this setting. In view of its technical advantages, BIOMED-2-GS could become the standard method for detecting T-cell clonality in CTCL.

Acknowledgments

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