Detection of Clonal T-Cell Receptor \(\gamma\) Gene Rearrangements in Early Mycosis Fungoides/Sezary Syndrome by Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis (PCR/DGGE)

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We used a gene amplification strategy to analyze T-cell receptor (TCR) gene rearrangements in 185 specimens, including mycosis fungoides/Sezary syndrome (MF/SS), other cutaneous neoplasms, inflammatory dermatoses, reactive lymphoid tissues, and normal skin. Genomic DNA was extracted from lesional tissues and rearrangements of the TCR-\(\gamma\) chain gene were amplified using the polymerase chain reaction (PCR) with primers specific for rearrangements involving V\(\gamma\)1-8 or V\(\gamma\)9 gene segments. The resulting PCR products were then separated according to their nucleotide sequence as well as size by denaturing gradient gel electrophoresis (DGGE). Dominant clonal TCR-\(\gamma\) gene rearrangements were detected in 61 of 68 MF/SS cases by PCR/DGGE. This sensitivity of 90% compared to a sensitivity of only 59% when dominant clonality was sought in 17 of these same cases by Southern blot analysis of TCR-\(\beta\) gene rearrangements. This difference in sensitivity was greatest in early, minimally infiltrated skin lesions. PCR/DGGE was also more sensitive than Southern blot analysis for detecting peripheral blood involvement in two cases of early MF. Among 12 additional specimens of suspected MF/SS, nine (75%) showed clonal TCR-\(\gamma\) gene rearrangements by PCR/DGGE including six of eight cases with a previously confirmed diagnosis of MF/SS and three of four cases without prior known MF/SS. Among 105 non-MF/SS specimens, dominant TCR-\(\gamma\) gene rearrangements were detected in only six cases (6%). Four were diagnosed as chronic dermatitis and two were diagnosed as cutaneous lymphoid hyperplasia.

We conclude that the large majority of MF/SS cases, including patch phase disease, possess dominant clonal TCR-\(\gamma\) gene rearrangements. PCR/DGGE is more sensitive than Southern blot analysis for detecting dominant clonality and staging disease in patients with a confirmed diagnosis of MF/SS. However, because PCR/DGGE is sensitive enough to detect dominant TCR-\(\gamma\) gene rearrangements in a subset of patients with chronic dermatitis, it cannot be used as the sole criterion for establishing a diagnosis of T-cell lymphoma. As with other molecular biologic clonality assays, clinicopathologic correlation is essential. Nevertheless, the detection of dominant clonality in some cases of histologically nonspecific dermatitis allows the identification of a previously unrecognized subset of patients, i.e., those with “clonal dermatitis.” It will be important to determine the long-term risk of MF/SS among these patients because our study indicated that MF/SS can sometimes present with lesions indistinguishable from clonal dermatitis. J Invest Dermatol 103:34-41, 1994

s in other types of non-Hodgkin’s lymphoma, the diagnosis of mycosis fungoides/Sezary syndrome (MF/SS) [1–4] has been aided by the detection of dominant clonal T-cell populations in biopsy specimens using Southern blot analysis of T-cell receptor (TCR) gene rearrangements [1,5,6]. Nevertheless, several features of this approach limit its usefulness [7]. These limitations include the requirement for at least 10^8-10^6 cells for a single analysis, an interval of about one to two weeks necessary to complete the analysis, and frequent reliance on radiolabeled hybridization probes—all of which contribute to costs and overall inconvenience. Most importantly, the sensitivity limitations of Southern blot analysis make the analysis of lesions containing sparse lymphocytic infiltrates problematic because the tumor cell density in these specimens is often too low to ensure against false negative results.

In an effort to address some of these limitations, various molecular biologic alternatives to routine Southern blot analysis of TCR gene rearrangements have been devised. Many of these methods have been reviewed individually elsewhere [7]. They generally involve the amplification of TCR-\(\beta\) or TCR-\(\gamma\) gene rearrangements

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using polymerase chain reaction (PCR) techniques. In contrast to Southern blot analysis, which utilizes the configuration of rearranged V, D, and J gene segments as clonal markers, PCR-based methods utilize nucleotide sequence at the junction of rearranged segments as markers of clonality. Unique sequences at these sites are created during the process of gene rearrangement due to the combination of short stretches of random nucleotides between the segments prior to joining. The result is a novel sequence specific for an individual lymphocyte and any clonal progeny that may derive from that cell.

Although each PCR-based method has certain advantages over Southern blot analysis, most possess limitations of their own, involving one or more of the following factors: the need for working with RNA, which is more labile than DNA, the need for labor-intensive cloning and sequence analysis of TCR gene rearrangements, and/or the need for radioisotopes and autoradiography. However, one method avoids these limitations. This method involves the amplification of TCR-gene gene rearrangements followed by the fractionation of the resulting PCR products by denaturing gradient gel electrophoresis (DGGE) [8]. The entire procedure has been referred to as PCR/DGGE analysis. It involves three main steps: 1) extraction of genomic DNA from patient specimens, 2) PCR-based amplification of TCR-gene gene rearrangements from the genomic DNA, and 3) fractionation of the resulting PCR products by DGGE.

In principle applicable to any TCR gene, the TCR-gene is the best target for analysis by PCR/DGGE for several reasons. First, compared to other TCR genes, TCR-g is a relatively simple gene, consisting in the germline form of eight V gene segments, seven V pseudogene segments, and five J segments, of which Vy2-Vy5, Vy8-Vy11, Jy1, and Jy2 frequently participate in rearrangements [9-12]. Furthermore, there is enough nucleotide sequence homology among various V-region and J segments to allow the design of a small number of consensus primers capable of amplifying most TCR-gene gene rearrangements [8-12]. Because the Vy and Jy target sequences complementary to that of the primers of rearranged TCR-gene genes are less than 500 bp apart within rearranged genes, genomic DNA can be used as the PCR substrate. This obviates the need for reverse transcription of mRNA, which generally has been used for the PCR-based amplification of TCR-gene gene rearrangements because the standard genomic Vβ and Cβ target sequences lie too far apart for efficient amplification [7].

DGGE was initially developed for the analysis of point mutations because it separates double-stranded DNA fragments according to nucleotide sequence. If sufficient numbers of fragments have identical size and sequence, as is produced by PCR of TCR genes from the genomic DNA of a clone of T cells, they form a band visible under ultraviolet light after staining of the gel with ethidium bromide. This obviates the need for reverse transcription of mRNA, which is more labile than DNA, the need for labor-intensive cloning and sequence analysis of TCR gene rearrangements, and/or the need for radioisotopes and autoradiography. However, one method avoids these limitations. This method involves the amplification of TCR-gene gene rearrangements followed by the fractionation of the resulting PCR products by DGGE.

Southern Blot Analysis of TCR Gene Rearrangements DNA extraction, Southern blot hybridization, and autoradiography were performed using standard methods [18] with minor modifications. All MF/SS cases studied were obtained from patients with cutaneous MF/SS, and 105 patients with other diseases. The non-MF/SS specimens included lymphoid infiltrates and included nonspecific dermatitis (23 cases), cutaneous squamous cell carcinoma (20 cases), cutaneous basal cell carcinoma (10 cases), cutaneous B-cell lymphoma (10 cases), cutaneous lymphoid hyperplasia (nine cases), hyperplastic tonsils (three cases), psoriasis (three cases), pityriasis rubra pilaris (two cases), acne vulgaris (one case), and basal cell carcinoma (two cases). Southern blots were also stained additionally with anti-TCR-P antibody RA3. The minimum panel for all other MF/SS, cutaneous lymphoid hyperplasia cases included the MF/SS panel plus CD19, CD20, CD35, and immunoglobulin chains (kappa, lambda, mu, delta). In each case studied, the immunohistologic findings were characteristic for MF/SS, B-cell lymphoma, or cutaneous lymphoid hyperplasia as described previously [1]. In addition, all cases of cutaneous lymphoid hyperplasia and cutaneous B-cell lymphoma were characterized by Southern blot analysis of immunoglobulin heavy-chain gene rearrangements using a Jg probe as described previously [17]. A monoclonal pattern was observed in each case of B-cell lymphoma but not in any case of lymphoid hyperplasia.

PCRs Genomic DNA (2 µg) was suspended in PCR buffer containing 40 pmole of each of two oligonucleotide primers, 200 µM (each) of the four deoxyribonucleoside triphosphates, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 0.01% gelatin, and 2.5 units Taq DNA polymerase in a total volume of 100 µl [8]. The thermocycling conditions consisted of denaturation at 94 °C for 1 min and annealing at 55 °C for 1.5 min, and extension at 72 °C for 1.5 min. Initially 25 cycles were performed using a "outer" Vy and Jy consensus primers corresponding to conserved Vy and Jy sequences. A 10-µl portion of first-round PCR products in fresh reagent buffer was then used as a target in a subsequent round of PCR using a set of "inner" Vy and Jy consensus primers corresponding to conserved Vy and Jy sequences nested inside the sequences complementary to the outer primer pair. The outer and inner primer pairs used for the amplification of rearrangements involving Vy1-8 are listed in Table I. For the amplification of rearrangements involving Vy9, the outer and inner Vy8 primers were replaced by the single Vy9 primer also listed in Table I. First round Vy1-8 PCR products were approximately 480 bp, whereas second round Vy1-8 PCR products were approximately 1700 bp.

Materials and Methods

Tissues Representative samples of skin, lymph node, and/or peripheral blood mononuclear cells were obtained from 185 cases: 68 patients with MF/SS, 12 patients with suspected MF/SS, and 105 patients with other diseases. The non-MF/SS specimens included lymphoid infiltrates and included nonspecific dermatitis (23 cases), cutaneous squamous cell carcinoma (20 cases), cutaneous basal cell carcinoma (10 cases), cutaneous B-cell lymphoma (10 cases), cutaneous lymphoid hyperplasia (nine cases), hyperplastic tonsils (three cases), psoriasis (three cases), pityriasis rubra pilaris (two cases), acne vulgaris (one case), and basal cell carcinoma (two cases). Southern blots were also stained additionally with anti-TCR-P antibody RA3. The minimum panel for all other MF/SS, cutaneous lymphoid hyperplasia cases included the MF/SS panel plus CD19, CD20, CD35, and immunoglobulin chains (kappa, lambda, mu, delta). In each case studied, the immunohistologic findings were characteristic for MF/SS, B-cell lymphoma, or cutaneous lymphoid hyperplasia as described previously [1]. In addition, all cases of cutaneous lymphoid hyperplasia and cutaneous B-cell lymphoma were characterized by Southern blot analysis of immunoglobulin heavy-chain gene rearrangements using a Jg probe as described previously [17]. A monoclonal pattern was observed in each case of B-cell lymphoma but not in any case of lymphoid hyperplasia.

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were approximately 420 bp. First- and second-round Vy9 PCR products were slightly smaller, measuring about 400 bp and 380 bp, respectively. Representative second-round Vy1-8 PCR products are illustrated in Fig. 1. Selection of optimal primer sequences and PCR conditions including the first-round/second-round strategy were determined empirically in prior studies using T-cell lines [8]. All PCR reactions in this study were performed using a Thermal Cycler (Perkin-Elmer, Norwalk, CT). DNA from each specimen was amplified at least twice to ensure that any band detected was likely to represent an actual clonal population rather than the chance amplification of a product from a single cell [8]. Negative controls included PCR reactions containing all reagents except target DNA. Because our primers are specific for Vy1-9 and Jy1-2, they do not allow amplification of rearrangements involving other TCR-γ gene segments such as Vy10, Vy11, and other Jγ segments.

DGGE DGGE was performed in an apparatus similar to one currently available from CBS Scientific Co., Inc. (Del Mar, CA) following procedures described by Myers et al. [15]. PCR-amplified genomic TCR-γ rearrangements were separated by electrophoresis at 60°C through a 6.5% polyacrylamide gel containing a 30–60% urea/formamide denaturing gradient as described previously [8]. The gels were then stained by immersion in 1 μg/ml ethidium bromide for 15 min, after which gels were photographed under 300 nm UV light. To eliminate the presence of faint, nonspecific bands sometimes noted in prior studies of polyclonal T-cell samples, an additional step of melting the PCR products at 95°C for 5 min and then reannealing at 60°C for 1 h was introduced prior to loading the samples onto the gels [8]. This resulted in diffuse smears without discrete bands in lanes containing polyclonal TCR-γ PCR products (Fig. 2).

Technical Points Concerning PCR/DGGE Two important technical points emerged from these studies. First, cases containing monoclonal T-cell gene rearrangements showed dominant clonal bands for Vy1-8 (Fig. 3). Second, cases containing polyclonal T-cell gene rearrangements sometimes noted in prior studies of polyclonal T-cell samples, an additional step of melting the PCR products at 95°C for 5 min and then reannealing at 60°C for 1 h was introduced prior to loading the samples onto the gels [8]. This resulted in diffuse smears without discrete bands in lanes containing polyclonal TCR-γ PCR products (Fig. 2).

**Table 1. TCR-γ Primer Combinations**

<table>
<thead>
<tr>
<th>PCR Round</th>
<th>Vγ Primer</th>
<th>Jγ Primer</th>
</tr>
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<tbody>
<tr>
<td>First round</td>
<td>Vγ1-8 “Outer”</td>
<td>Jγ “Outer”</td>
</tr>
<tr>
<td>18</td>
<td>5’ GAA GCT TCT AGC TTT CCT GTC TC 3’</td>
<td>40</td>
</tr>
<tr>
<td>or Vγ9</td>
<td>5’ CGT CCA CAA GTG TTG TCT CAC 3’</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>5’ GGA ATT CCA AAT TCT TGG TT TTT A A 3’</td>
<td></td>
</tr>
<tr>
<td>Second round</td>
<td>Vγ1-8 “Inner”</td>
<td>Jγ “Inner”</td>
</tr>
<tr>
<td>43</td>
<td>5’ CTC GAG TGC GCT GCC TAC AGA GAG G 3’</td>
<td>67</td>
</tr>
<tr>
<td>or Vγ9</td>
<td>5’ GGA TCC ACT GCC AAA GAG TTT CTT 3’</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers above nucleotide sequences designate the bp position of the complementary end nucleotides within the target gene. Underlined nucleotides represent restriction endonuclease digestion sites as follows: outer Vγ1-8 (HindIII), outer Jγ1 (SalI), inner Vγ1-8 (XhoI), inner Jγ1 (BamHI), Vγ9 (EcoRI).
populations, as evidenced by Southern blot analysis of TCR-β gene rearrangements showing only one or two rearranged bands, can exhibit more than two discrete bands by PCR/DGGE. Often this appears as one or two strong bands and one or more weaker bands. At present, the source of these additional weaker bands is unknown; however, they are not due to the final melting/reannealing step performed prior to loading denaturing gradient gels [8]. Some of these bands may be due to heteroduplexes arising from annealing together of single strands of DNA amplified from different clonal TCR-γ gene rearrangements. More likely, these bands reflect an oligoclonal T-cell background from which a dominant T-cell clone has emerged. Because of possible differences in the lesional density of these clones, only the most dominant one may be detectable by Southern blot analysis. Recently, we have used elution, cloning, and sequencing to show that the comigrating dominant bands present in paired PCR/DGGE analyses of specimens of CD30+ large cell lymphoma and lymphomatoid papulosis obtained from a single patient do in fact represent a monoclonal TCR-γ gene rearrangement shared by both lesions [20].

Second, although the PCR/DGGE band position produced by a given sample is constant in different lanes of the same gel, it can vary somewhat among different gels. This is probably due to subtle technical differences that can occur with each separate PCR/DGGE analysis. Some degree, this issue can be addressed by electrophoresis in each gel of several standard fragments. Obviously, the best approach to this problem is that specimens to be compared should be run side-by-side in the same gel, as represented in Fig 3. We found matching dominant clonal band patterns in four MF/SS cases in which multiple specimens were analyzed by PCR/DGGE.

RESULTS

Analysis of MF/SS Specimens To determine whether PCR/DGGE analysis can reliably detect the presence of dominant T-cell clonal populations in skin specimens considered to contain histologic features diagnostic of MF/SS, we analyzed tissues from 68 cases. Sixty-one of these (90%) showed discrete bands in PCR/DGGE analyses including 16 Vγ1-8Vγ9+, 35 Vγ1-8Vγ9− and 10 Vγ1−8Vγ9− cases. Examples are illustrated in Fig 3. The presence of both Vγ1−8 and Vγ9 rearrangements in a single case is due presumably to rearrangement of both TCR-γ gene alleles. The seven specimens that failed to show discrete bands showed only diffuse smears in PCR/DGGE analyses of both Vγ1−8 and Vγ9 rearrangements.

In 17 of the 68 cases, Southern blot analysis of TCR-β gene rearrangements was also performed on the same tissues. Representative Southern blot results are illustrated in Fig 4, and a comparison between the results of TCR-γ PCR/DGGE and TCR-β Southern blot analysis is presented in Table II along with details concerning the type of specimens analyzed. Dominant clonality was detected by PCR/DGGE analysis in six of seven cases that were negative by Southern blotting. Table III compares the false-negative rates for these two molecular biologic assays in early versus advanced MF/SS. These data demonstrate that PCR/DGGE is more sensitive than Southern blot analysis for detecting dominant TCR gene rearrangements in early lesional tissues (patches and thin plaques). Specific examples are illustrated in Fig 5.

We chose to compare PCR/DGGE analysis of TCR-γ gene rearrangements to Southern blot analysis of TCR-β gene rearrangements because Southern blot analysis of TCR-γ gene rearrangements can sometimes produce false-positive results [21,22], because the TCR-β gene is the one that has been studied by Southern blot analysis most commonly in the published literature [7], and because almost all cases of MF/SS exhibit TCR-β gene rearrangements and express the TCR-αβ heterodimer [23]. This was also true of the cases studied by Southern blot analysis in this series because they reacted with anti-TCR-β monoclonal antibody 8A3. Southern blot analysis of TCR-γ gene rearrangements was performed in two of these MF/SS cases as well as in two cases each of psoriasis, normal skin, and reactive lymphoid tissues, and one case each of granuloma annulare and cutaneous B-cell lymphoma. None of these specimens exhibited detectable monoclonal TCR-γ gene rearrangements.

Analysis of Suspected MF/SS Specimens In 12 additional cases, we performed PCR/DGGE analyses on skin specimens judged to contain possible MF/SS but to show features insufficient for an unequivocal histologic diagnosis. Eight of these specimens came from patients with a prior confirmed diagnosis of MF/SS. Whether or not there was a prior diagnosis of MF/SS, there was a strong correlation between dominant clonality and histology suspicious for MF/SS. Dominant clonality was found in 75% of these patients (six of eight) with a history of MF/SS, and in the same percentage of these patients (three of four) without a history of MF/SS.

In four of the cases with prior MF/SS, diagnostic specimens containing neoplastic T cells (blood in three cases and a skin tumor in the fourth) were available for comparison with the non-diagnostic specimen from the same case. PCR/DGGE showed similar bands in the paired diagnostic specimens in three of these cases. The fourth showed discrete bands in both specimens of the pair (skin patch and tumor), although the bands were found in different positions within the gel. The precise explanation for this phenomenon is obscure, but changes in clonal TCR-γ gene rearrangements over time have been observed in cases of acute lymphoblastic leukemia. In this situation, such changes have been attributed to derivation of the leukemia from a transformed lymphocytic precursor, with subsequent rearrangement of TCR-γ genes in progeny cells [17].

Overall, these cases demonstrated that in patients with a past history of MF/SS, the disease can recur as a clonal, histologically nondiagnostic, cutaneous T-cell infiltrate. These cases also sug-
Figure 4. Southern blot analysis of TCR-β gene rearrangements in some of the MF cases illustrated in Fig 3. One or two rearranged bands (arrowheads) are present in each lane consistent with the presence of a monoclonal T-cell population in each case. Unmarked germline bands are also present in each lane. BamHI germline band is approximately 23 kb. BglII germline bands cluster around 9 kb. The number of germline bands in BglII digests varies because of a polymorphism in the number of restriction sites. Lanes 1–4, same case as in Fig 3, lanes 1–6. Lanes 1 and 2 are BglII digests of skin and lymph node, respectively. Lanes 3 and 4 are BamHI digests of skin and lymph node, respectively. Lanes 5 and 6, BglII and BamHI digests, respectively, of the same case as in Fig 3, lane 8. Lanes 7 and 8, BglII and BamHI digests, respectively, of the same case as in Fig 3, lane 9. Lanes 9 and 10, BglII and BamHI digests, respectively, of the same case as in Fig 3, lanes 15 and 16. Lane 11, BglII digest of same case as in Fig 3, lanes 19 and 20. This last case was the only one in our series that showed dominant clonality by Southern blot analysis but not by PCR/DGGE.

Table II. Results of TCR-γ PCR-DGGE and TCR-β Southern Blot Analysis as a Function of Specimen Type in MF/SS

<table>
<thead>
<tr>
<th>TCR-β Southern Blot</th>
<th>PCR/DGGE+</th>
<th>PCR/DGGE−</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>9(1B,1T,5Q,2P)</td>
<td>1(Q)</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>6(1T,1Q,4P)</td>
<td>1(P)</td>
<td>7</td>
</tr>
<tr>
<td>Not done</td>
<td>46(5B,10T,31P)</td>
<td>5(2B,3P)</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>7</td>
<td>68</td>
</tr>
</tbody>
</table>

* B, leukemic blood; T, cutaneous tumor; Q, cutaneous thick plaque; P, cutaneous patch/thin plaque.

Figure 5. Southern blot analysis showing an absence of detectable TCR-β gene rearrangements in MF cases that showed dominant TCR-γ gene rearrangements by PCR/DGGE. Lane 1, negative Southern blot analysis of lesional skin from patch-type MF (TCR-β probe, BglII digest). Lanes 2 and 3, PCR/DGGE analysis of the same case as in lane 1 shows a Vγ1-8+ pattern in morphologically normal peripheral blood (lane 2) and a Vγ1-8− pattern in the same skin specimen as in lane 1 (lane 3). Lane 4, negative Southern blot analysis of lesional skin from a MF tumor nodule (TCR-β probe, BglII digest). Lanes 5 and 6, PCR/DGGE analysis of the same specimen as in lane 4 shows a Vγ1-8+ and Vγ9-8+ pattern, respectively. The difference in the number of germline bands visible in lanes 1 and 4 is due to a polymorphism in the presence of BglII restriction sites.

Molecular Biologic Staging of MF/SS by PCR/DGGE Analysis In addition to the information regarding tumor clone distribution obtained by PCR/DGGE analysis of multiple skin specimens as discussed above, we also studied extracutaneous tissues in four MF/SS cases. The peripheral blood of two stage Ib MF patients contained low numbers of circulating atypical lymphocytes but no rare Sézary cells. TCR-β and TCR-γ Southern blot analysis of these blood specimens were negative; however, both specimens were clonal by PCR/DGGE. A third patient with stage Ib MF and a cytologically normal peripheral blood smear lacked evidence of blood involvement from nonspecific dermatitis. Because patients with lymphomatoid papulosis, a clonal, clinically benign disorder, are at increased risk for MF/SS and Hodgkin's disease [1,24,25], it was not unexpected to observe an association between overt lymphomas and this clonal, nonspecific dermatosis. Although this patient's skin lesions cleared transiently in response to total skin electron beam radiation therapy, they recurred and proved refractory to subsequent topical and systemic therapy. The patient eventually died of sepsis. An autopsy was not performed.

Table III. False Negative Rates for TCR-γ PCR/DGGE and TCR-β Southern Blot Analysis in Early versus Advanced MF/SS

<table>
<thead>
<tr>
<th>Method</th>
<th>Early MF/SS</th>
<th>Advanced MF/SS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR/DGGE</td>
<td>1/7 (14%)</td>
<td>1/10 (10%)</td>
<td>2/17 (12%)</td>
</tr>
<tr>
<td>Southern Blot</td>
<td>5/7 (71%)</td>
<td>2/10 (20%)</td>
<td>7/17 (41%)</td>
</tr>
</tbody>
</table>

* Patches or thin plaques. 
* Thick plaques, tumor, or leukemic blood.
volvement by PCR/DGGE although her cutaneous MF patch lesions were positive. Similarly, a cytologically normal lymph node removed from a patient with stage Ia MF also lacked evidence of involvement by PCR/DGGE although her cutaneous MF patches showed dominant clonality.

**Analysis of Non-MF/SS Specimens** To determine the clonality of TCR-γ gene rearrangements in non-T-cell lymphoma specimens, we used PCR/DGGE to analyze the 105 biopsies of inflammatory dermatoses, cutaneous B-cell lymphoma, normal skin, and reactive lymphoid tissues listed in *Materials and Methods*. In the vast majority of cases (94%), Vy1-8 and Vy9 PCR products produced only a diffuse smear after DGGE, consistent with the absence of dominant T-cell clonal populations bearing Vy1-9/Vy1-2 rearrangements in these samples (Fig 2). This included the cases of cutaneous squamous cell carcinoma and basal cell carcinoma selected for study based on the presence of a significant T-cell host response as assessed by immunohistology.

Ocult dominant TCR-γ gene rearrangements were detected in only six specimens, including four cases of nonspecific dermatitis (hereafter referred to as "clonal dermatitis") and two cases of cutaneous lymphoid hyperplasia (hereafter referred to as "clonal cutaneous lymphoid hyperplasia"). The nonspecific dermatitis group contained 23 cases characterized histopathologically by a superficial perivascular lymphocytic infiltrate accompanied in most cases by variable degrees of spongiosis, epidermal hyperplasia, and/or inflammation at the epidermal-dermal interface. Overall, this dermatitis group contained one case with clonal Vy1-8 rearrangements and three cases with clonal Vy9 rearrangements. The cutaneous lymphoid hyperplasia group contained nine cases, including one case with clonal Vy1-8 rearrangements and one case with clonal Vy9 rearrangements.

In the six cases of clonal dermatitis and clonal cutaneous lymphoid hyperplasia, the Vy1-8 and Vy9 PCR products formed reproducible discrete bands after DGGE; however, in a number of these cases, the bands obtained from Vy9 PCR products were rather faint. In general, amplification with the Vy9 primer is more likely to produce artifactual bands of low intensity because the number of TCR-γ gene rearrangements containing a Vy9 segment in a polyclonal population of T cells will be fewer than those containing Vy1-8 segments. Consequently, a TCR-γ gene rearrangement present in only one copy may conceivably amplify to produce a visible band in the gel. On the other hand, the ability to obtain the same faint bands in multiple amplifications of DNA from the present specimens suggests that this is not a sufficient explanation and the bands observed do arise from dominant T-cell clones in the tissues.

**DISCUSSION**

In this study, we used PCR/DGGE analysis to determine the clonality of T cells in samples obtained from 185 individuals including 105 non-T-cell lymphoma patients representing a wide variety of diagnoses, 68 patients with MF/SS, and 12 patients with suspected MF/SS. Our findings indicate that the large majority of MF/SS cases (90%) contain clonal TCR-γ gene rearrangements involving Vy1-9 and Vy1-2. Only seven of 68 MF/SS cases (10%) lacked detectable dominant clonal TCR-γ gene rearrangements. Presumably these cases contained TCR-γ rearrangements involving Vy genes not detectable with our primers, previously deleted Vy1-9 rearrangements, or germline TCR-γ genes. Because there are at least two other known TCR-γ V segments (Vy10 and Vy11) [11], it is probable that the future use of PCR primers capable of amplifying rearrangements of these other Vy segments will result in an overall sensitivity exceeding the current 90% level. Use of primers to other Jγ genes (JγP, JγP1, and JγP2) may also increase the yield of positive results, although prior studies suggest that many TCR-γ gene rearrangements in T-cell neoplasms involve Jγ1 or Jγ2, which are both detected by our Jγ consensus primers [8-11]. The relatively large proportion of MF/SS cases containing dominant TCR Vy9 gene rearrangements may be explained at least in part by the apparent preferential generation and selection of TCR-γ gene rearrangements involving 3′ V region genes such as Vy9 [26,27]. This may also explain the predominance of dominant TCR Vy9 gene rearrangements among the six cases of clonal non-MF/SS specimens. It should be noted that despite negative findings in our cases of cutaneous B-cell lymphoma, the detection of dominant clonal TCR-γ gene rearrangements cannot be used to infer T-cell lineage because some B-cell neoplasms can exhibit clonal TCR-γ gene rearrangements [11].

In this series of cases, which included mostly early skin lesions containing relatively sparse infiltrates, PCR/DGGE was considerably more sensitive than Southern blot analysis for detecting dominant clonal TCR-γ gene rearrangements in early MF/SS. Comparable levels of sensitivity for detecting clonality in MF/SS have been described in preliminary reports by others using related methods [Stäb G, Mielek V, Grieser H, Menke M, Duller B, Sterry W: PCR-analysis of T-cell-receptor (TCR)-γ genes to differentiate between malignant and reactive T-cell infiltraters of the skin (abstr). *J Invest Dermatol* 100:458, 1993; van Oostven JW, Bakels V, Meijer CJLM, Willemze R: Comparisons of Southern blot and PCR/DGGE analysis of T-cell receptor gene rearrangement analysis in the diagnosis of cutaneous lymphomas (abstr). *J Invest Dermatol* 100:458, 1993; Greiner TC, Jaffe E: Analysis of clonal TCR-gamma gene rearrangements in paraffin embedded T cell lymphoid neoplasms by denaturing gradient gel electrophoresis of GC clamped PCR products (abstr). *Lab Invest* 68:91, 1993]. Consequently, PCR/DGGE has considerable value as a backup assay for lymphoma specimens that are negative by Southern blot analysis. As detailed in Table II, six of 17 MF/SS cases studied by both Southern blot and PCR/DGGE were shown to contain dominant T-cell clones only by PCR/DGGE. In many of these cases, the specimens analyzed were minimally infiltrated lesions characteristic of early disease (Tables II and III). These findings indicate that PCR/DGGE will be superior to Southern blot analysis for staging MF/SS patients and monitoring their response to therapy. This conclusion is also supported by the ability of PCR/DGGE to identify the MF/SS dominant clonal pattern in histologically equivocal specimens from known MF/SS patients and its superior sensitivity in our initial staging investigations.

In addition, these findings are consistent with the view that histologically recognizable patch-type MF is a monoclonal disorder. They suggest that the failure of Southern blot analysis to detect monoclonality in some prior studies of patch-type MF [28,29] was due to the limited sensitivity of this technique rather than to the absence of a monoclonal tumor cell population within the lesions. The increased sensitivity of PCR/DGGE analysis relative to Southern blot analysis may derive both from its applicability to smaller absolute numbers of lymphocytes and from its ability to detect a clonal fraction of lymphocytes about tenfold lower than Southern blotting [8].

Our current data also offer insights into the clonality of T-cell populations in normal skin, reactive lymphoid tissues, a wide variety of inflammatory dermatoses, and the host response to tumors. In the vast majority of these non-MF/SS specimens (99 of 105 or 94%), we were able to detect only polyclonal TCR-γ gene rearrangements, supporting the polyclonal nature of T cells in these samples. Nevertheless, six of our 105 non-T-cell lymphoma specimens (6%) contained evidence of dominant T-cell clonality. It is possible that the detection of discrete bands by PCR/DGGE in these six cases might have arisen from the fortuitous amplification of TCR-γ genes in over-represented reactive T-cell clones; however, it is also possible that the dominant TCR-γ gene rearrangements in some or all of these cases originated in occult neoplastic clones. It is noteworthy that the six clonal non-MF/SS cases were restricted to two general disease categories: nonspecific dermatitis (four cases) and cutaneous lymphoid hyperplasia (two cases). We felt it was important to study both of these disease categories because early MF/SS is sometimes difficult to distinguish from chronic der-
mattis, and other cutaneous lymphomas are sometimes difficult to distinguish from cutaneous lymphoid hyperplasia [1–4].

A subset of cutaneous lymphoid hyperplasia cases have been shown previously to contain occult dominant B-cell or T-cell clones [1,30–34], and it is known that clonal TCR-γ gene rearrangements can be present in both B-cell and T-cell lymphoma [1,5–7,11]. Therefore, it is possible that the two clonal cases of cutaneous lymphoid hyperplasia each harbored an occult neoplastic B-cell or T-cell clone bearing a TCR-γ gene rearrangement. Furthermore, these same studies indicate that patients with clonal cutaneous lymphoid hyperplasia represent a recognizable subgroup that is at increased risk for the development of cutaneous B-cell lymphoma.

In addition to clonal cutaneous lymphoid hyperplasia, there are other situations in which a clinically benign chronic disease has been associated with an increased risk of overt lymphoma. These include other forms of extranodal lymphoid hyperplasia, the so-called monoclonal gammapathies, lymphomatoid papulosis and angioimmunoblastic lymphadenopathy with dysproteinemia [24,25,35–37]. This relationship between clonal B-cell and T-cell processes and increased lymphoma risk suggests that patients with clonal dermatitis may also represent a subgroup that is at increased risk for the development of clinically overt lymphoproliferative disorders such as MF/SS. This conclusion is supported by our data, which showed that previously undiagnosed MF/SS can present as apparent clonal dermatitis (see case J.I. above), and that a strong correlation exists between dominant clonality and histologic features suspicious for MF/SS. This conclusion is also consistent with numerous anecdotal reports of MF/SS developing at sites of apparent chronic dermatitis [3,4]. It is these observations that may account in part for the difficulty pathologists have encountered in developing minimal morphologic criteria for the diagnosis of early MF/SS, and the relatively high level of inter- and intra-observer variability involved in early MF/SS diagnosis [1,2,3,8]. When considering the potential neoplastic significance of clonal dermatitis, it is also important to note that non-neoplastic lesions of allergic contact dermatitis, induced in two patients with active MF, did not contain dominant TCR-γ gene rearrangements detectable by PCR/DGGE although their MF lesions did [Veeken H, Sklar J, Wood GS: Presence of malignant T cells in lesions of allergic contact dermatitis developing in patients with cutaneous T cell lymphoma (abstr). Immunobiol 186:152, 1992].

In summary, our findings demonstrate that clonal TCR-γ gene rearrangements are present in the large majority of MF/SS patients. This indicates that MF/SS is a clonal disorder even in its earliest recognizable phases. Furthermore, PCR/DGGE is more sensitive than Southern blot analysis of TCR gene rearrangements for detecting dominant clonality and staging disease in patients with confirmed MF/SS. However, because PCR/DGGE is sensitive enough to detect clonal TCR-γ gene rearrangements in a subset of patients with chronic dermatitis, it cannot be relied upon as the sole criterion for establishing an unequivocal diagnosis of T-cell lymphoma. As with other molecular biologic assays of lymphoid clonality, clinicopathologic correlation is essential. Nevertheless, the detection of dominant clonality in some cases of histologically nonspecific dermatitis allows the identification of a previously unrecognized subset of patients, i.e., those with “clonal dermatitis.” It will be important to determine the long-term risk of MF/SS among these patients because our study indicates that MF/SS can sometimes present as apparent clonal dermatitis. Close follow-up of these patients could allow the diagnosis of lymphoma to be made at the earliest possible time. This is important because some studies have suggested that appropriate therapy initiated early in the course of MF/SS may improve not only the initial complete response rate but also long-term freedom from relapse and possibly the chances for cure [39–41]. Furthermore, early stage of disease at the time of diagnosis is a consistently favorable prognostic variable associated with longer median survival regardless of the specific type of treatment given [39–41].

**References**


25. Fagioli M, Ciccone E, Bottino C, Bulini F, Grignani F, Moretta A, Moretta L, Pellici PG: The Cy1-encoded disulfide-linked and the Cy2-encoded non-di-
sulfite-linked forms of the γ/δ heterodimer use different γ and δ variable regions. Blood 76:279–284, 1990


ERRATA

In the April issue, in the Review by Francesco Sinigaglia (Sinigaglia F: The molecular basis of metal recognition by T cells. J Invest Dermatol 102:398–401, 1994) under the heading Metal Recognition by T Cells Is MHC Restricted, the fourth sentence should read “The results were clear: the clones responded to the nickel in association with HLA-DRw11 [7] and to gold in association with DR1 [9] or DR4 (Romagnoli P, Sinigaglia R, unpublished).” Also in that issue, in the report by Amagai et al (Amagai M, Kärpätä S, Klaus-Kovtun V, Udery MC, Stanley JR: Extracellular domain of pemphigus vulgaris antigen (desmoglein 3) mediates weak homophilic adhesion. J Invest Dermatol 102:402–408, 1994), the last sentence of the third paragraph should read “α-catenin, on the other hand, is sometimes difficult to detect [13,15]” and the first sentence of the following paragraph should read “The cytoplasmic tail of PV A, like the other desmogleins, binds only to plakoglobin (which co-migrates on sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) with α-catenin, but is probably a distinct protein [17,10]) and does not bind to α- or β-catenin [19].”