Correlations Between Clinical, Histologic, Blood, and Skin Polymerase Chain Reaction Outcome in Patients Treated for Mycosis Fungoides

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Little information is currently available regarding post-treatment outcome of TCR-targeted PCR in skin and/or peripheral blood in patients with Mycosis Fungoides (MF) when a dominant gene rearrangement is present at time of diagnosis. To address this matter, a study evaluating the correlations between post-treatment clinical, histological, blood and skin PCR data was conducted in MF patients. Twenty-seven MF patients with dominant gene rearrangement in skin lesions at time of diagnosis were selected. Peripheral blood samples were investigated as well before treatment and post treatment molecular data in skin and blood were compared with clinical and histological outcome. A dominant gene rearrangement was detected before treatment in blood of 16/25 patients. The dominant gene rearrangement disappeared from cutaneous lesions in 8/13 patients displaying complete clinical and histological response whereas skin PCR remained positive in all 10 patients with histologically persistent disease. A dominant gene rearrangement was still present in blood in 10/16 patients after treatment and blood data were not correlated with skin molecular response. This study confirms frequent detection of a dominant gene rearrangement in peripheral blood in MF patients and shows that PCR may remain positive in lesional sites even when skin lesions are successfully treated.

Key words: Mycosis Fungoides; post-treatment outcome; TCR gene rearrangement. J Invest Dermatol 121:614–617, 2003

Molecular biology methods using mainly T cell receptor (TCR)-targeted amplification by polymerase chain reaction (PCR) currently allow the detection of the presence of a dominant gene rearrangement in cutaneous lesions, but also in peripheral blood in a high proportion of patients with cutaneous T cell lymphomas, even at the early patch or plaque stages of the disease (Weiss et al, 1985; Weiss et al, 1989; Wood et al, 1994; Bachelez et al, 1995; Bakels et al, 1992; Veelken et al, 1995; Dommann et al, 1996; Curcio et al, 1997; Muche et al, 1997; Fraser-Andrews et al, 2000). A positive PCR in peripheral blood of patients with cutaneous T cell lymphomas, however, might not be related to the presence of tumoral cells in all cases but rather to the age of the patients (Delfau-Larue et al, 2000). Conversely, few data are currently available regarding the evolution of PCR data in skin lesions and peripheral blood after treatment. Also, the possible correlations that can be established between clinical and histologic results of the treatment on one hand, and the outcome of PCR data in skin and/or peripheral blood on another hand have been poorly studied (Andrews et al, 2001). Finally, the predictive value of post-treatment PCR data as regards to the overall outcome of the disease has only been seldom investigated. Accordingly, we designed a prospective study investigating the outcome of PCR data both in skin lesions and, if applicable, in peripheral blood in patients treated for mycosis fungoides (MF) and displaying a dominant gene rearrangement in skin lesions before treatment. Furthermore, the relationship between post-therapy PCR status and therapeutic results evaluated on clinical and histologic grounds was analyzed.

MATERIALS AND METHODS

Patients and samples Twenty-seven patients with MF were selected for this study, all of them fulfilling the following criteria: clinical cutaneous lesions consistent with the diagnosis before treatment; typical histologic features, including band-like subepidermal mononucleated infiltrate with epidermotropism and occasional atypical cells with convoluted nuclei; large predominance of CD4+ cells among the infiltrating lymphocytes; dominant gene rearrangement in skin lesions as disclosed by the PCR-based method described below. Patients with multiple clonal bands (digoclonal pattern) were excluded from the study in an effort to suppress any confusing factor in the subsequent interpretation of PCR data. The mean age of patients was 55.4 y old (ranging from 22.3 to 76.5) at the time of diagnosis and sex ratio (M/F) was 2:1. These 27 patients were staged according to the usual classification of MF as follows: seven Ia, 18 Ib, and two IIa. For all patients, clinical, histologic, immunologic, and PCR data were obtained before and 6 to 12 wk after the end of the treatment if the latter was of limited duration or at least 6 mo after the therapy was implemented in the case of a more protracted, chronic treatment. The mean interval of time between pretreatment and post-treatment evaluation was 8.3 mo (ranging from 5.2 to 14.4 mo). More precisely, treatment efficiency was evaluated on clinical (disappearance or persistence of initial lesions), histologic, and immunologic (multiple biopsies performed on sites close to areas where a specific infiltrate was present before treatment, processed for histologic and immunohistochemical study) grounds, whereas post-treatment PCR was...
performed both in peripheral blood and in cutaneous areas as close as possible to sites where it was positive before treatment. The therapies consisted in psoralen + ultraviolet A therapy (10 patients), oral retinoids (two patients), or both (four patients), weekly intramuscular injections of methotrexate (two patients), total body electron beam therapy (three patients), or in topically applied BCNU (six patients).

PCR amplification of TCR gene encoding TCR-γ chain
Genomic DNA was extracted from peripheral blood and skin samples using the QIAamp DNA Mini Kit (Qiagen, Les Ulis France) for TCR analysis. Two multiplex-PCR were performed using TCR-γ V1 to V8, V9, V10, V11, and fluorescent Jγ1/Jγ2 or fluorescent Jγ3/Jγ5 consensus primers allowing identification of virtually almost all known Vγ/Jγ combinations. Amplification was performed using 250 ng of genomic DNA as template, together with 6.25 pmol of each primer, 200 μM of each deoxyribonucleoside triphosphate, 2.5 mM of MgCl₂ and 0.625 U Taq polymerase (AmpliTaq, Perkin Elmer, Les Ulis France) in a total volume of 25 μL. PCR amplifications were performed using a Techne Genius Thermocycler (Techne, Cambridge, UK) with the following conditions: denaturation at 94°C during 5 min followed by two cycles of denaturation at 94°C (30 s), annealing at 61°C (30 s), extension at 72°C (30 s), followed by two cycles of denaturation at 94°C (30 s), annealing at 60°C (30 s), extension at 72°C (30 s), followed by 26 cycles of denaturation at 94°C (30 s), annealing at 59°C (30 s), extension at 72°C (30 s) and followed by final extension period of 10 min at 72°C.

Sizing analysis of PCR products
Electrophoresis was carried out in an ABI 373A DNA sequencer (Applied Biosystems, Les Ulis, France) with the following conditions: denaturation at 94°C for 1 min followed by two cycles of denaturation at 94°C (30 s), annealing at 59°C (30 s), extension at 72°C (30 s) followed by two cycles of denaturation at 94°C (30 s), annealing at 60°C (30 s), extension at 72°C (30 s), followed by 26 cycles of denaturation at 94°C (30 s), annealing at 59°C (30 s), extension at 72°C (30 s) and followed by final extension period of 10 min at 72°C.

Statistics
Owing to the small size of subsets, statistical tests using nonparametric methods (Mann–Whileoxon–Whitney tests) were used to assess the significance of the relationship between clinical and histologic response on one hand and PCR data outcome, either in skin or in blood, on another hand and to compare the percentage of cases where PCR products of different sizes were obtained from skin and peripheral blood samples, according to the stage of the disease.

RESULTS
Clinical, histologic, immunologic, and PCR (both in skin and blood) data were obtained for all patients before and after treatment was implemented, except for two of them for peripheral blood PCR.

Initial peripheral blood data
Pretreatment analysis of peripheral blood displayed a positive PCR in 16 of 25 investigated patients (64%) (four of six stage Ia, 10 of 17 stage Ib, two of two stage Ila). In three cases, the sizes of the amplified fragments were different in skin and blood samples as visualized on comigration analysis (two stage Ib, one stage Ila). This increased rate of PCR, products size discordance between skin and peripheral blood in stage Ib was statistically significant when compared with stage Ia but the limited size of samples actually precluded any definite conclusion.

Post-treatment data
Post-treatment clinical, histologic, immunologic, and PCR data were interpretable for all investigated patients (Table I). A complete (14 patients) or partial (eight patients, among which three showed <50% improvement) clinical response was obtained in 22 of 27 patients. Multiple biopsies showed a consistent disappearance of the cutaneous lymphocytic CD4+ infiltrate in 17 cases (13 and four with complete and partial clinical response, respectively), whereas three displayed a partial result (one with complete clinical remission and two with partial clinical improvement).

Post-treatment PCR was negative in skin lesions in only eight patients (30%) (three stage Ia, four stage Ib, one stage Ila) all of them in complete clinical and histologic remission cases. Conversely, it was still positive with amplified fragments of identical size in the remaining 19 patients, including five stage Ib patients for whom a complete clinical and histologic remission was obtained.

Peripheral blood PCR became negative in six of 16 cases (37.5%) (two stage Ia, three stage Ib, one stage Ila), including four patients with complete clinical and histologic responses, whereas PCR remained positive in blood in 10 patients of whom four achieved a complete clinical and histologic remission (three stage Ib, one Ila). PCR was still positive in cutaneous lesions in four of the six patients with negative post-treatment PCR in blood (three stage Ib, one Ila), whereas it remained positive in peripheral blood in spite of skin molecular response in two of eight patients (one stage Ib, one stage Ila). One patient with stage Ila disease and negative post-treatment PCR in skin but persistent positive PCR in blood, along with a complete clinical and histologic clearance of lesions, relapsed within 9 mo after the remission was completed. He experienced reappearance of plaques and return of a positive PCR in skin lesions with an amplified fragment of the same size as before treatment.

Overall, there was no significant statistical difference as regards skin or peripheral blood PCR outcome between patients clinically responding, either partially or completely, and nonresponding to treatment (p = 0.27). Conversely, a negative post-treatment PCR in skin samples was significantly more frequent in patients displaying both complete clinical and histologic response than in patients with partial, dissociated or no clinical and histologic response (p = 0.02) but this was not true for peripheral blood PCR data (p = 0.35).

Table I. Clinical, histologic, and molecular post-treatment outcome of 27 patients with MF displaying an initial dominant T cell clone in skin lesions

<table>
<thead>
<tr>
<th>Patients</th>
<th>Detection of a dominant T cell clone in PB</th>
<th>Clinical response</th>
<th>Histologic response</th>
<th>Molecular response in skin (and PB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Ia</td>
<td>4/6</td>
<td>C: 4</td>
<td>C: 3</td>
<td>3/3 (2/2)</td>
</tr>
<tr>
<td>18 Ib</td>
<td>10/17</td>
<td>P: 3</td>
<td>A: 3</td>
<td>0/3 (0/1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C: 9</td>
<td>P: 4</td>
<td>C: 1</td>
</tr>
<tr>
<td>2 Ila</td>
<td>2/2</td>
<td>A: 5</td>
<td>A: 2</td>
<td>0/5 (0/2)</td>
</tr>
</tbody>
</table>

PB, peripheral blood; C, complete; P, partial; A, absence.
DISCUSSION

An increasing number of studies have recently demonstrated that a TCR-targeted PCR is frequently positive in peripheral blood in cutaneous T cell lymphomas patients including early stages of the disease (Weiss et al, 1989; Bakels et al, 1992; Veelken et al, 1995; Dommann et al, 1996; Curco et al, 1997; Muche et al, 1997; Fraser-Andrews et al, 2000). Molecular data, including direct sequencing or comparison of migration patterns of the amplified fragments in privileged cases, favor an identity between dominant T cell populations in skin and peripheral blood. The question of the specificity of a positive PCR in peripheral blood, however, has been raised in other cases, all the more because a dominant gene rearrangement seems to be more frequently present in elderly patients without definite disease (Delfau-Larue et al, 2000). On another hand, recent studies have clearly established that the presence of a dominant T cell population in peripheral blood (and possibly in lymph nodes) was an independent unfavorable prognostic factor in patients with MF, whether or not the same dominant population was present in skin lesions (Bakels et al, 2001; Beylot-Barry et al, 2001; Scarsbric k et al, 2001). The same tendency seems true when PCR is positive in skin lesions, as a study has established that disease stage and positive PCR in skin were both independent factors related to a worse prognosis in patients with MF (Delfau-Larue et al, 1998a).

Our series confirms that a positive PCR may be found in peripheral blood in a high percentage of patients with MF as it was present in 64% of patients. More specifically, PCR was positive in 14 of 23 patients during the early stages (Ia and Ib) and in two of two patients with stage IIa. The sizes of amplified sequences from blood and skin were identical in 13 of 16 patients where PCR was positive in both compartments, indirectly suggesting the presence of identical T cell dominant populations in skin and peripheral blood, perhaps originating from the same neoplastic clone. The method we used (single-color PCR), however, was unable to identify bands that look identical in size but may correspond to different gene rearrangements, and is unlikely to tell apart with full accuracy PCR products that are 1 or 2 base pairs different in size. Accordingly, a sequencing of the amplified sequence would be required to unambiguously establish such an identity. These data are different from the figures published by Beylot-Barry et al (2001) who found that an identical clone was present both in skin and peripheral blood in only 25% of patients with MF (17 of 67); however, these authors used a different method, based on GG-clamp primers and denaturing gradient gel electrophoresis, which theoretically allows an accurate assessment of both size and sequence of PCR products. Eventually, the higher percentage of discordance in size between amplified fragments obtained from skin or blood samples in more advanced clinical stages is probably due to the presence of a distinct clonal population and an increase in the number of patients with a different gene rearrangement. Therefore, it is doubtful that the therapeutic aim in MF should be to detect PCR positivity in the blood, especially in early stage patients, but this trend needs confirmation by large-scale studies. Conversely, the value of peripheral blood data appears to be much more confusing with different outcomes of the cutaneous dominant gene rearrangement and of its blood counterpart, if present, in a number of patients; therefore, this study does not support the usefulness of investigating peripheral blood PCR data in the follow-up of stage I and II MF patients.

Eventually, no clear relationship between the type of treatment and PCR outcome was obvious but the size of the sample was small for each different therapeutic subset. In particular, total body electron beam therapy, which is considered a major and highly effective therapy, did not result in the disappearance of the dominant gene rearrangement in the three patients submitted to this treatment, but two of them were affected with more advanced disease (stage IIa). Conversely, four of 10 patients treated with psoralein + ultraviolet A displayed negative PCR in skin samples after treatment was completed.

This relatively frequent persistence of a dominant gene rearrangement either in skin or in blood or both in patients with MF even when the disease looks perfectly controlled by the treatment is a new finding but its clinical relevance is questionable. These results might provide a valuable explanation for the frequent occurrence of relapses even when a complete clinical and histologic remission is achieved. Their significance is unclear, however, as the true prognostic value of a positive PCR in skin and/or peripheral blood after treatment as regards long-term outcome of the disease would require a large-scale prospective study. Furthermore, it is doubtful that the therapeutic aim in MF should shift toward obtaining a negative PCR both in skin and blood (when positive before treatment) in all cases, as it would probably lead to unnecessarily aggressive and toxic therapies. Such an attempt to reach a molecular clearance in lesions and peripheral blood, however, might be worthwhile in selected cases such as late stage or multirelapsing MF when the prevention of recurrences may be of major interest.

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