T-Cell Clonality in Pityriasis Lichenoides et Varioliformis Acuta

A Heteroduplex Analysis of 20 Cases

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Background: Cutaneous lesions of pityriasis lichenoides et varioliformis acuta (PLEVA), a T cell–mediated cutaneous inflammatory condition, are clinically similar to lymphomatoid papulosis (LyP), leading some authors to hypothesize that they are part of the same spectrum of lymphoproliferative disorders, although reports of the development of cutaneous lymphoma in patients with PLEVA are not as frequent as they are for patients with LyP. Furthermore, unlike in cases of LyP, no systematic search for a dominant T-cell clone has been carried out in cases of PLEVA, whereas clones have been detected in a few cases of PLEVA using mainly Southern blot analysis.

Objective: To investigate T-cell clonality in a series of archival PLEVA lesions.

Tissues: Archival paraffin-embedded biopsy specimens from 20 clinically and pathologically typical cases of PLEVA were selected.

Main Outcome Measure: Identification of a dominant T-cell clone by polymerase chain reaction and heteroduplex analysis targeted on the TCRγ gene. Peripheral blood mononuclear cells (PBMCs) and Jurkat cells were used as negative and positive controls. Serial dilutions of Jurkat T-cell lymphoma DNA in PBMC DNA were used to assess the sensitivity of the method.

Results: Analysis of 13 (65%) of 20 PLEVA biopsy specimens revealed the presence of a dominant T-cell clone. Positive and negative controls confirmed the specificity of the procedure. The sensitivity was determined to be between 1% and 5% of the total T-cell infiltrate.

Conclusions: This study provides further evidence for the presence of a dominant T-cell clone in skin lesions of some patients with PLEVA and supports the hypothesis that PLEVA is part of the spectrum of clonal T-cell cutaneous lymphoproliferative disorders.

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MATERIALS AND METHODS

PATIENTS AND TISSUES

Paraffin-embedded cutaneous biopsy specimens from 20 patients with PLEVA were selected from the University Hospital of Montpellier, France; there were 12 men and 8 women and their ages ranged from 6 to 42 years (mean ± SD, 26 ± 3 years). The cutaneous eruptions lasted for an average of 4.2 weeks before the biopsy was performed. In all cases, a diagnosis of PLEVA was made on the basis of the following features: (1) an eruption of inflammatory papules with a central crust and an occasional necrotic pattern leaving atrophic scars; (2) exocytosis, extravasation of erythrocytes, necrosis of scattered keratinocytes, basal layer vacuolization, and a dermal lymphohistiocytic infiltrate with a lichenoid and/or perivascular distribution without a significant presence of large lymphoid cells; and (3) predominance of CD2+CD3+CD8+ lymphocytes in the dermal infiltrate and little or no CD30 staining. Peripheral blood mononuclear cells (PBMCs) from healthy donors and paraffin-embedded tissues from 10 patients with clinically and histologically typical lichen planus were used as negative controls. Jurkat cells, a non-Hodgkin T-lymphoma cell line with a known monoclonal TCRg rearrangement, served as a positive control and for the assessment of the sensitivity of detection of clonal T cells by the heteroduplex method. A typical case of mycosis fungoides with a known clonal TCRg rearrangement was used as a positive control as well.

MOLECULAR ANALYSIS

Paraffin-embedded tissues were cut into 25-μm-thick sections that were dewaxed by xylene and ethanol according to standard protocols and digested overnight with 0.1 mg of proteinase K. Genomic DNA was extracted from Jurkat cells using a Tissueamp DNA extraction kit (Quiagen Inc, Santa Clara, Calif) according to the manufacturer’s instructions.

Four pairs of primers were used to amplify the V/J regions of the γ chain of the TCR according to previously published reports. The sequences of the primers are summarized in the Table. Each pair includes the same J consensus primer specific for the Jγ region of the TCRγ chain and a different Vγ primer depending on the targeted variable Vγ region: Vγ1 as a consensus primer for Vγ regions 1 to 8; Vγ9, Vγ10, and Vγ11 for Vγ regions 9, 10, and 11, respectively. Polymerase chain reaction was performed separately for each pair of primers on a DNA thermal cycler (N801-0150; Perkin-Elmer-Cetus, Norwalk, Conn), with 100 ng of genomic DNA, 100 pmol of each primer and Ready-to-Go PCR beads (Pharmacia Biotech, Piscataway, NJ; 200-μmol/L each dATP, dCTP, dTTP, and dGTP; 50-mmol/L potassium chloride; 1.5-mmol/L magnesium chloride; 10-mmol/L Tris-hydrochloride pH 9.0; 1.5 U Taq DNA polymerase) with a final volume of 25 μL. The samples were denatured at 95°C for 5 minutes and then amplified for 35 cycles of 50 seconds each at 95°C, 50 seconds each at 65°C, and 1 minute 30 seconds each at 72°C, followed by a final extension segment of 10 minutes at 72°C. The quality of the PCR was controlled by electrophoresis of a sample of 5 μL on a 2% agarose gel.

In heteroduplex analysis, PCR products are denatured at a high temperature and then slowly cooled to allow renaturation of the single strands. As random renaturation occurs, both homoduplexes (with identical junctional regions) and heteroduplexes (with different junctional regions) appear. In a polyclonal lymphoid population, heteroduplexes with internal secondary structures and loops will form, giving rise to a smear on migration in a non-denaturing gel. In a mixed lymphoid population of both monoclonal and polyclonal origins, homoduplexes originating from the monoclonal subset are likely to appear, mixed with heteroduplexes derived from polyclonal cells. These homoduplexes, devoid of loops, will migrate as usual double-stranded DNA according to their size and will appear as a clear band on a nondenaturing gel, which is usually associated with the polyclonal background smear derived from heteroduplexes. This method has been validated for cutaneous T-cell infiltrates when using products of a TCRγ-targeted PCR as a template. Briefly, 5 μL of the PCR mixture was mixed with an equal volume of denaturing buffer (93% formamide; 5% EDTA [0.5 mol/L; pH 8.0]; 0.25 g/L of xylene cyanol; and 0.25 g/L of bromophenol blue), and the resulting solution was heated at 95°C for 5 minutes for denaturation of the double-stranded PCR products. Heteroduplex formation was then allowed to proceed by random renaturation at 30°C for 1 hour. Samples were immediately cooled on ice for 5 minutes and loaded on a nondenaturing 12% polyacrylamide gel without glycerol. The samples were run at 4°C at 5 W for 16 to 20 hours in Tris-borate EDTA buffer (half diluted). Finally, the gels were read by UV transillumination after conventional ethidium bromide staining for 45 minutes and washing with distilled water for 15 minutes.

To assess the sensitivity of a dominant T-cell clone detection by this method, successive dilutions of Jurkat cell DNA in PBMC-derived DNA were used (ie, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:33, 1:50, 1:100, 1:200, 1:500, and 1:1000). The sensitivity threshold was then determined as the last dilution giving a visible band out of a smear of heteroduplex formation.

a true cutaneous lymphoma, although such an outcome has sometimes occurred in acute and chronic forms of pityriasis lichenoides. The host immune response could be represented by the usual predominance of CD8+ lymphocytes in the dermal infiltrate.

Only 4 patients have been studied with molecular methods, 3 with the rather insensitive Southern blot method, to determine the presence of a dominant T-cell clone that would be an essential feature of a lymphoproliferative disorder. All patients disclosed a dominant T-cell clone in the lesions, which seems to favor the hypothesis of a lymphoproliferative disorder, but the number of studied cases is too small to draw a definite conclusion. Therefore, we studied T-cell clonality in cutaneous lesions of 20 clinically and histologically typical cases of PLEVA from a university hospital using sensitive polymerase chain reaction (PCR) amplification followed by heteroduplex analysis of the TCRγ gene, a reliable and sensitive method to detect the presence of a dominant T-cell clone in a mixed cutaneous infiltrate. A dominant clone was found in 13 of 20 cases, which supports the hypothe-
Peripheral blood mononuclear cells and all cases of lichen planus displayed a polyclonal pattern with the 4 sets of primers. This was the expected result because lichen planus is a known polyclonal disease. Conversely, the DNA from Jurkat cells, a T-cell lymphoma line, showed a distinct band of the predicted size (300 base pairs [bp]) and had practically no background smear in the analysis of PCR products with the Vβ1-Jγ primers, whereas no band was present in the analysis of PCR products from the other sets of primers. These results were also expected because all the cells contained the same TCRγ gene rearrangement. The sensitivity assessment showed that a band was detectable up to a range of dilution between 1% and 5% Jurkat lymphoma cells in polyclonal DNA, which means that a T-cell clone can be detected by this method when it represents at least 5% of the T cells from which DNA has been extracted (Figure 1). The mycosis fungoides case displayed the expected monoclonal band as well.

Of the 20 PLEVA cases examined, 13 (65%) displayed a homoduplex pattern with a clear band of the expected size but of variable intensity (Figure 2), which is consistent with the presence of a dominant T-cell clone within the dermal lymphoid infiltrate. In all cases, this result was obtained when using the PCR products from the Vβ1-Jγ amplification, whereas only a polyclonal pattern was obtained when using the 3 other sets of primers. In all cases, these results were confirmed by a second round of PCR-heteroduplex analysis.

In this study, we searched for the presence of a dominant T-cell clone within the dermal lymphoid infiltrate in 20 cases of PLEVA using PCR-heteroduplex analysis to detect rearrangements of the TCRγ gene. We found that skin lesions from 13 (65%) of 20 cases disclosed a pattern consistent with the presence of a dominant clone, whereas negative and positive controls showed the expected results. Cases of LyP were excluded by strict pathologic and immunophenotypic criteria. The possibility of contamination by a single clonal sample was excluded by direct sequencing of the monoclonal bands, which displayed a unique sequence in each case (data not shown). It is possible that the actual percentage of PLEVA cases with a dominant T-cell clone is even higher, since the sensitivity of the heteroduplex method was only about 5% in this study, which is similar to the data in the literature.20

There are several possible interpretations for our findings. One is that PLEVA is a cutaneous lymphoproliferative disorder with a theoretical malignant potential. This hypothesis is supported by the rare development of malignant lymphomas in patients with PLEVA.12-15 Another possibility is that PLEVA is closely related to LyP, which clearly has a malignant potential and was a clonal T-cell disorder in most cases.21 This hypothesis is supported by the similar clinical presentations and the possible occurrence of both PLEVA and LyP in the same patient.10,11 However, in most patients PLEVA and LyP seem to have different histopathologic and immunophenotypic features, with PLEVA lacking the frequent CD30+ large atypical cells characteristic of LyP7,21,22 and having a predominant CD8+ infiltrate at the dermoepidermal junction that is generally lacking in LyP.7 Still another explanation for our findings is that the dominant T-cell clone in PLEVA represents a clonal immunologic response to an unknown antigen or infectious agent.2,9,23-25

We favor the hypothesis that PLEVA is part of the clonal T-cell cutaneous lymphoproliferative spectrum, such as small plaques parapsoriasis,26 as previously suggested.8 The rare occurrence of lymphoma during the course of PLEVA and, in most cases, a spontaneous regression after several months, suggests that a vigorous host immune reaction controls and eventually eliminates the T-cell clone. Additional genetic alterations, which remain to be defined, may be required for the infrequent progression of PLEVA into malignant lymphoma. As an example, we have demonstrated that the progression of

**Table 1:** Primers for the TCRγ-Targeted Polymerase Chain Reaction

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<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Vγ1</td>
<td>5′-TACATCCACTGATCAACCTGCA-3′</td>
</tr>
<tr>
<td>Vγ9</td>
<td>5′-TACATCACTGCTGTCGGCTG-3′</td>
</tr>
<tr>
<td>Vγ10</td>
<td>5′-CCGACGAGTTAGAAGAC-3′</td>
</tr>
<tr>
<td>Vγ10/11</td>
<td>5′-CAGCATGGGTAAGACAAGC-3′</td>
</tr>
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| Jγ1/2   | 5′-CCCCTCGACTACCTGGAAGATGTTGATTCTC-3′   

**Figure 1.** Heteroduplex analysis of successive dilutions of Jurkat lymphoma cell DNA in peripheral blood mononuclear cell DNA (molecular weight [mw] ladder [arrow, 300 base pairs]; lane 1, peripheral blood mononuclear cells; lane 2, Jurkat cells; lane 3, mycosis fungoides; lanes 4-15, increasing ratio of Jurkat cell DNA in peripheral blood mononuclear cell DNA [1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:33, 1:50, 1:100, 1:200, 1:500, and 1:1000]). Disappearance of the monoclonal pattern occurred between 1:50 and 1:100.

**Figure 2.** Heteroduplex analysis of polymerase chain reaction products of pityriasis lichenoides et varioliformis acuta cases (molecular weight [mw] ladder [arrow, 300 base pairs]; lane 1, mycosis fungoides; lanes 2-6 and 8, pityriasis lichenoides et varioliformis acuta cases; lane 7, Jurkat cells. There was a monoclonal pattern for samples corresponding to lanes 2-4, 6, and 8.
the T-cell clone in LyP is associated with the loss of growth regulation by transforming growth factor β because of mutations within the transforming growth factor β receptor complex.27,28

In this study, 10 of 13 PLEVA patients with a dominant T-cell clone experienced a usual clinical course with spontaneous disappearance of skin lesions after several months, whereas 3 cases evolved toward a more chronic form but without any evidence of a lymphoid malignancy after a follow-up of at least 2½ years. In conclusion, our study gives further support to the hypothesis that PLEVA may be in some, if not most cases, a clonal T-cell cutaneous lymphoproliferative disorder.

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REFERENCES