Clonotypic Heterogeneity in Cutaneous T-Cell Lymphomas

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ABSTRACT

The antigen receptor genes studied (immunoglobulin gene for B-cells, and T-cell receptor -ß or -y gene for T-cells) represent the most powerful tools for diagnosing the clonality of a lymphoid lineage. We have clonally typed 23 cutaneous T-cell lymphomas and 5 were found to be clonotypically heterogeneous. Analysis of each patient was performed either from skin biopsies taken over several months apart or from different tumor samples. In these cases, T-cell lymphoma clonotypic heterogeneity was demonstrated and was especially evident when examining different tumor sites. Moreover, in one case, a biogenotypic population (immunoglobulin and T-cell receptor-rearranged) was found. This unexpected high frequency of T-cell clonal heterogeneity (22%) could be explained either by the evolution of subclones from a single undifferentiated malignant cell or by the independent transformation to cancer of 2 or more lymphocytes, though the latter seems less likely. Clonotypic heterogeneity seems to be frequent in T-cell lymphomas with cutaneous lesions as in B-cell leukemias.

INTRODUCTION

The evidence that human hematological cancers are in fact clonal hematopoietic stem cell disorders emerged essentially from cytogenetic and isoenzyme studies (1). Receptor gene rearrangement studies are the most potent tool for assessing the clonality of a lymphoid lineage, specifically the Ig genes in B-cells (2) and the TCR gene in T-cell lymphoproliferations (3, 4).

Clonotypic heterogeneity is the existence, in one patient, of different immunogenotypes that define different clones, the derivation of which (from one malignant stem cell or from multiple malignant cells) being unknown.

In some patients, DNA analyses of malignant lymphoid tissues obtained from different sites or at various times show different clonal rearrangements of Ig or TCR genes. In these patients, the individual tumors appear to develop as completely independent clones. Conversely, other patients have various lymphoma populations that are derived from a single malignant transformation event, although they have different Ig gene patterns and thus appear to be biclonal (5). Although there are numerous examples of different clonal rearrangements of the Ig gene, different clonal rearrangements of TCR genes have been thought to be rare. In a prospective study of cutaneous lymphomas using Southern blot analysis, an unexpectedly high frequency of various clonal rearrangements of TCR genes at different sites was observed. We report here the immunogenotype of 5 patients with this clonotypic heterogeneity and discuss the significance of this finding.

MATERIALS AND METHODS

Patients. Specimens taken from different tumor sites from 23 cutaneous T-cell lymphoma patients were subjected to molecular analyses. All patients had clinical manifestations of cutaneous lymphomas and the diagnoses were confirmed morphologically by microscopic examination. Five of them (22%) exhibited clonotypic heterogeneity. The diagnosis, organ origin of the tumor specimens, and probes used in the study of these 5 patients are summarized in Table 1. Cases 1 and 2 had 2 skin biopsies at intervals of 3 and 5 months, respectively. Patients 1 and 3 had clinical features of acute leukemia. Patients 2 and 4 presented, respectively, a parapsoriasis "en plaque" and large plaques of mycosis fungoides with lymph node enlargement.

Histopathological Studies. All biopsy specimens were evaluated by one of the authors (Y. Fonck). Monoclonal antibodies CD3, CD4, CD8, CD1, CD2, CD20, CD19, and CD21, labeled with immunoperoxidase, were used, in some cases, for immunohistological study.

DNA Studies. Skin biopsy specimens and peripheral blood were obtained from the 23 patients. Also, 3 patients (cases 1, 4, and 5) had a lymph node biopsy. Skin biopsies and surgically removed lymph nodes were directly submerged in liquid nitrogen and then stored in a nitrogen atmosphere. Skin and lymph node samples were pulverized with a French’s press, then transferred into a Tris-EDTA buffer containing 0.02% collagenase. For analysis of DNA from peripheral blood, the monoclonal fraction was collected directly after lysis in Tris-EDTA buffer. DNA was extracted from specimens and purified by standard procedures (6, 7); 10 μg of purified DNA was digested with both EcoRI and HindIII restriction enzymes, and the resulting fragments were size-fractionated by electrophoresis in 0.7% agarose gels, and then transferred onto nylon membranes (Zetaprobe; Bio-Rad) as described by Southern (8). DNA was hybridized with α-32P-radiolabeled DNA probes, in 50% formamide for approximately 18 h at 42°C. The filters were washed in 2 X standard saline citrate (2 x 15 min at room temperature) then in 0.1 X standard saline citrate, 0.1% sodium dodecyl sulfate (2 x 15 min at room temperature, 1 x 15 min at 65°C) and autoradiographed at ~80°C (Hyperfilm; Amersham). Probes used were (see Table 1): (a) TCR ß probe, a 770-base pair fragment from YT35 (9, 10) for the T-cell receptor-ß genes; (b) Jy probe, PH60 (11), a 700-base pair Hind III-Eco RI fragment containing the Jy1 segment for the human TCR -y-chain gene; and (c) JH probe, M13C76R51A (12) for the immunoglobulin heavy-chain gene.

RESULTS

Molecular studies performed on skin biopsies, lymph nodes, blood samples, and bone marrow from 23 patients with cutaneous T-cell lymphomas revealed clonotypic heterogeneity in 5 cases. Results of the DNA hybridization in these 5 cases with a TCR ß, TCR -y, and JH probes are shown in Table 1.

Case 1. This patient was in leukemic phase with skin and lymph node involvement (Tables 2 and 3). Four different genotypes were obtained with the 4 specimens from involved tissues (peripheral blood, first skin biopsy, second skin biopsy, and lymph node). DNA of the peripheral blood was in germinal configuration with all probes, although cytological examination revealed an abundance of malignant cells; however, these were different from those found in the lymph node. The DNA of the lymph node was in germinal configuration with the JH probe.
Table 1 Patients, diagnosis, tumor specimens, hybridization probes used, gene configuration, and size of the rearranged bands (kb)

<table>
<thead>
<tr>
<th>Patient diagnosis</th>
<th>Tumor specimens</th>
<th>TCR β probe</th>
<th>TCR γ probe (PH60)</th>
<th>JH probe (M13C76R51A)</th>
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<tbody>
<tr>
<td></td>
<td>EcoRI</td>
<td>HindIII</td>
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<td>1</td>
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<td></td>
<td></td>
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</tr>
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<td>g</td>
<td>g</td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>g</td>
<td>g</td>
<td>r(5.4)</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>g</td>
<td>r(5.4)</td>
<td>r(3.7;4.3)</td>
</tr>
<tr>
<td></td>
<td>LN</td>
<td>r(2.4)</td>
<td>r(2.4)</td>
<td>r(25;30)</td>
</tr>
<tr>
<td>2</td>
<td>MF</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>PB</td>
<td>g</td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>r(8;7;8;3;7)</td>
<td>g</td>
<td>r(5.4;7;9.5)</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>r(9)</td>
<td>r(5.4)</td>
<td>r(9.5)</td>
</tr>
<tr>
<td>3</td>
<td>NHL</td>
<td>PB</td>
<td>r(3.5)</td>
<td>r(3.4)</td>
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<tr>
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<td>S</td>
<td>r(3.6;9.2;7)</td>
<td>r(3.4)</td>
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<td>r(2.2)</td>
<td>r(2.9)</td>
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<td>PB</td>
<td>r(11)</td>
<td>g</td>
<td>g</td>
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<tr>
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<td>S</td>
<td>r(2.2)</td>
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* NHL, non-Hodgkin's lymphoma; SS, Sézary syndrome; MF, mycosis fungoides; S1, first skin biopsy; S2, second skin biopsy; PB, peripheral blood; LN, lymph node; BM, bone marrow; g, germinal configuration of the gene; r, rearranged configuration of the gene; ND, not done.

Table 2 Histopathological studies

<table>
<thead>
<tr>
<th>Case</th>
<th>Skin</th>
<th>Lymph node</th>
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<tbody>
<tr>
<td></td>
<td>CD3</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
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</table>

* Quantification: 0, no positive cells; 1, 0–25%; 2, 25–50%; 3, 50–75%; 4, 75–90%; 5, >75%.

Table 3 Immunophenotype of peripheral blood

<table>
<thead>
<tr>
<th>Case</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD20</th>
<th>HLA-DR</th>
<th>CD5</th>
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<td>11</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>93</td>
<td>85</td>
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</tr>
<tr>
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<td>66</td>
<td>4</td>
<td>73</td>
<td>1</td>
<td>10</td>
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<td>91</td>
<td>87</td>
<td>5</td>
<td>95</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

but was rearranged with the 2 TCR probes, indicating a monoclonal T-cell population. The 2 skin biopsies had the same TCR γ rearrangement corresponding to a clonal T-cell population different from that of the lymph node. In addition, only the second skin biopsy showed a rearrangement with the JH probe signifying one B-cell monoclonal population (Figs. 1 and 2).

Case 2. Two skin biopsies S1 and S2 were performed 3 months apart, and had a common T-cell population that was characterized using 2 probes. First, the TCR β probe gave bands of 8 kb and 5 kb with Eco RI and HindIII, respectively. Second, a Vγ11 rearrangement was picked up with PH60 (9.5 kb Eco RI and 5.6-kb HindIII bands) as described by Forster et al. (13). Whereas the biopsy S2 was monoclonal and displayed a unique T-cell population, the biopsy S1 performed 3 months earlier was clearly bidonal, with the additional T-cell population being rearranged on both TCR β alleles (Fig. 3).

Case 3. A common monoclonal T-cell population was observed in the peripheral blood, bone marrow, and skin (Fig. 4) of this patient. This clone had a rearranged TCR β2 gene. In the skin biopsy, a second monoclonal T-cell population was observed that had a rearranged TCR β1 gene. According to the restriction map of the TCR β gene with Eco RI and HindIII, any TCR β1 gene rearrangement appears to be in germinal configuration if the DNA is digested by HindIII and hybridized with a constant region gene probe. This was confirmed by BamHI digestion of the DNA: the first clone corresponds to a 20-kilobase rearranged band, the second to the 10-kilobase and 5.2 rearranged bands. Hybridization with the PH60 probe...
found only a common Vγ10 rearrangement in T-cells from the 3 tumor sites. Hybridization with a radiolabeled PUC18 probe excluded a contamination artifact.

Case 4. A partial report of this case has already been published (14). Hybridization of DNA to the TCR β and γ probes (Fig. 5) showed the same monoclonal T-cell population in the lymph node and skin biopsies. This population, which as a Vγ7 rearrangement, is different from that found in the peripheral blood as confirmed by probes to both the β and γ genes. Additionally in this patient, the monoclonal T-cell population detected in the peripheral blood did not come from bone marrow.

Case 5. The hybridization with the TCR β probe (Fig. 6; Table 1) showed that there was one monoclonal T-cell population with a rearranged TCR β1 gene in the skin; the same population was found in the peripheral blood with a second monoclonal T-cell population that had a rearranged TCR β2 gene.

We looked for a correlation between the clinical, histological, immunological, or immunohistological investigations (see Tables 2 and 3) and molecular analysis. No such relationship was found at any level except for patient 1 in which: (a) the 2 skin biopsies, taken 3 months apart, were histologically and genotypically different; (b) the immunophenotype of the second skin biopsy described between 25 and 50% B-cells, which are monoclonal by molecular genotyping, but about 50% of the cells in the lymph node were B-cells; however, these were not monoclonal; and (c) a confirmation of the genotypes, 3 different immunotypes were found in the skin, lymph node (with a reasonable level of CD2-positive cells), and peripheral blood with 79% CD3 and 1% CD2 cells (Table 3). In patient 3, the monoclonal population CD1+, CD2+ found in the peripheral blood and
bone marrow, and also in the skin of that patient, is probably the one with the TCR β2 gene rearranged population (Fig. 4). The population CD3+ CD4+, found in the skin but not in the peripheral blood or bone marrow, corresponds to the monoclonal population with TCR β1 gene rearrangement. For patient 5, although the immunotypes of the lymph node and skin were the same, no TCR β gene rearrangement was found in the lymph node even when genotypic analysis included multiple lymph node fragments. It is possible that either the pathological involvement was partial and we had not studied the involved portion of the lymph node with the 2 techniques, or less probably the lymph node genotype represented an undifferentiated malignant population that was confined to that tissue.

In all cases, it was impossible to determine T-monoclonality without genotypic analysis.

DISCUSSION

The high percentage of observed T-cell clonotypic heterogeneity (5 cases of 23 studied: 22%) was unexpected as were dual genotypes in cutaneous T-cell lymphomas (15) (autoradiography artifacts were eliminated by multiple endonuclease digests). This heterogeneity is perhaps one genotypic characteristic of cutaneous T-cell lymphomas.

Clonotypic heterogeneity has been previously reported in B-cell lymphoproliferative disorders (2, 16, 17), on the basis of immunoglobulin gene rearrangement analysis. For example, there may be 2 different types of Richter’s syndrome (18, 19): one characterized by 2 different B-cell clones and another that remains monoclonal and from which the B-non-Hodgkin’s lymphoma arises following the transformation of a preexisting malignant B-cell clone (20). Sklar et al. (21) estimated that the incidence of biclonal B-cell lymphoma may be as high as 10% of all B-cell lymphomas and even 25% in acute lymphoblastic leukemia (22). Biclonal B-cell lymphomas were described at different tumor sites in a single patient (23–25) or at different times of the evolution of the disease (22, 26, 27).

Dual genotype (15, 28, 29) and clonotypic heterogeneity have been less commonly described in T-cell cancers. Feller et al. (30) described a Lennert’s T-cell lymphoma in which a second lymphoma biopsy, performed 2 years after the first one, exhibited a rearrangement pattern that probably involved 3 alleles and was completely different from the first biopsy. In cutaneous T-cell lymphoproliferation, Weiss et al. (31) described one case of lymphomatoid papulosis in which different clones were present in 3 separate lesions on the same patient. More than 2 rearrangements of DNA have also been described in 2 cases of Sézary syndrome (32) and in the dermatopathic lymph node of one patient with mycosis fungoides (4).

Spreading of malignant cells in mycosis fungoides was demonstrated as starting the skin, then involving the lymph nodes, and finally spreading to the peripheral blood (33). So, circulating malignant cells in cutaneous T-cell lymphomas may have a prognostic significance (34). Nevertheless, histological differences between mycosis fungoides and Sézary syndrome lymph nodes suggested that different pathogenetic mechanisms may be operative in the development of these 2 conditions (35). In this study, we have not observed a common rearrangement pattern between the malignant population from a lymph node and that of the peripheral blood. This casts some doubt upon the relationship between lymph node involvement and circulating malignant cells. In patient 4, the follow up was too short (2 years) to make any conclusive remarks on the prognostic significance of the circulating malignant population that thereby did not necessarily correspond to a later stage.

We could not detect any prognostic difference between the 18 monoclonal cutaneous T-cell lymphomas and the 5 clonotypically heterogeneous cutaneous T-cell lymphomas, but the average follow up was too short (only about 2 years) for any meaningful conclusions to be drawn.

With regard to this long-term evolving disease, 2 patients (cases 1 and 3) had a nonepidermotropic cutaneous T-cell lymphoma (360). It is noteworthy that these 2 patients initially presented with severe clinical symptoms similar to acute lymphoblastic leukemia with skin involvement, never responded to a heavy chemotherapy regimen, and died quickly (2 and 4 months, respectively, after diagnosis). Serodiagnosis and molecular detection of human T-cell lymphotropic virus type 1 genome were negative in both cases.

Several mechanisms might explain the occurrence of clonotypic heterogeneity in non-Hodgkin’s lymphomas (28).

First is the evolution of subclones within a single malignant cell population. Raffeld et al. (25) reported cytogenetic evidence of monoclonality in contrast to genotypic heterogeneity in B-cell lymphomas. The emergence of a subclone can occur spontaneously, reflecting the multiple chromosome translocations known to occur in malignant T-cells, but can also represent a chemotherapeutically-induced clone (37, 38). For instance, a B-cell clone emerged in the second skin biopsy of patient 1, and a second T-cell clone arose in patient 5’s peripheral blood and in the skin of patient 3. This indicates that either a single transformed progenitor spawned both proliferating clones or that independent transformation events occurred in 2 related
cells carrying a common rearrangement of the TCR β gene. Nevertheless, treatment can also induce the disappearance of a monoclonal population (27) without the concomitant appearance of an immature phenotype, as occurred in patient 2. Malignant T-cells have been reported with both a single rearranged β-chain gene or 2 rearranged β-chain genes. In the case of a single rearranged gene, it may be possible for the nonrearranged gene to subsequently undergo rearrangement, producing subclones with 2 rearranged genes as in case 5. Alternatively, the clone of origin of a T-cell lymphoma may be an immature T-cell that has not yet rearranged any T-cell receptor gene. Multiple gene rearrangements may then be found among the more mature progeny of this cell. This mechanism might explain all 5 of our cases, especially case 1, in which the most immature T-cells were found in peripheral blood without TCR gene rearrangement, whereas more mature cells were located in the skin and lymph node.

Second is the rare coincidence in which 2 or more lymphocytes independently transform to a malignant state or less simultaneously (exemplified by patients 1 and 4). This implies that some lymphomas arose through a progression from the creation of an oligoclonal preneoplastic pool of lymphoid cells with the subsequent conversion of a single subclone. Recent reports provide strong evidence for the presence of more than one leukemic cell clone as well in B-cell lymphoproliferations at first presentation (16, 39) as in T-cell leukemogenesis induced by human T-cell lymphotropic virus type I (40).

A third mechanism seems to be involved in one case (patient 1) in which clonal rearrangement was noted with both JH and TCR β probes. In this case, the lymphoma cells might be derived from a single transformed early lymphocytic precursor. Lympocyte-committed stem cells capable of generating both B- and T-cells are presumed to exist, although the evidence accumulated to date for such a precursor is indirect (41). A second hypothesis may be related to non-specificity at the level of the recombinase enzyme that directs the process of DNA rearrangement (42). The emergence of a B-cell population in the skin in 3 months raised the possibility that this clone could have emerged from a polyclonal B-cell population directed against the malignant T-cell population.

Cumulative data from our patients indicate that these 3 mechanisms could coexist in the genesis of T-cell lymphomas. The most probable mechanism seems to be the emergence of multiple rearrangements from an unarranged undifferentiated malignant stem cell.

However, a definitive demonstration of this in humans will be difficult, even by coupling the various available techniques of clonality studies. Actually, cytogenetically independent cell populations can arise from a common progenitor in individual patients 4 (43). X chromosome inactivation analysis by RFLP can be applied only in women and has value when the 2 clones have a different inactivation pattern, (otherwise there is a 50% chance that 2 different clones will have the same active parental allele). This latter techniques could be applied to patients (women with different clones). RFLP is useful in hematological disorders (44), however, it is inappropriate in blood samples with a low level of malignant involvement or in skin samples with high concentrations of inflammatory cells that are immunologically indistinguishable from the malignant cells (Southern blots would always show the 2 X-RFLP).

Clonotypic heterogeneity in T-cell lymphomas seems to be caused by the same mechanisms (25) and to be as frequent as B-cell leukemias. Additionally, although our 5 patient’s tumors exhibited some resistance to therapy, further characterization of cutaneous T-cell lymphomas will be needed to evaluate the prognostic value of clonotypic heterogeneity.

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REFERENCES

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