Genotypic Analysis of Cutaneous T-Cell Lymphomas

Elisabeth Ralfkiaer, M.D., Nigel T. J. O’Connor, M.R.C.P.,* Julie Crick, B.Sc., Gunhild L. Wantzin, M.D., Ph.D., and David Y. Mason, D.M.

Departments of Pathology (ER) and Dermatology (GLW), The Finsen Institute, University of Copenhagen, Copenhagen, Denmark, and the MRC Molecular Haemotology Unit (NTJO’C, JC) and Department of Haemotology (DYM), John Radcliffe Hospital, University of Oxford, Oxford, U.K.

The gene encoding the beta-chain of the T-cell antigen receptor (TCR) has been analyzed for evidence of rearrangement in skin, blood, and lymph node specimens from 23 cases of known or suspected cutaneous T-cell lymphoma (CTCL). Two cutaneous large cell lymphomas, 4 cases of Sézary syndrome, and 5 cases of advanced (tumor) stages of mycosis fungoides showed clonal rearrangement of the TCR beta-chain gene in all samples, including lymph nodes in which histologic examination revealed only dermatopathic lymphadenitis. These results indicate that DNA analysis provides a valuable means for improving the diagnosis of extracutaneous disease in advanced stages of CTCL. In contrast, the gene was in a germline configuration in all samples from 12 patients with plaque stages of mycosis fungoides or suspected early CTCL, suggesting that in these 2 conditions the T-cell proliferation is either polyclonal or contains very few monoclonal (i.e., neoplastic) cells. J Invest Dermatol 88:762–763, 1987

T

he early recognition of cutaneous T-cell lymphomas (CTCL) constitutes a difficult task for both clinicians and histopathologists, and much effort has been directed towards the search for alternative, diagnostic criteria, e.g., ultrastructural [1], cell kinetic [2,3] or immunophenotypic [4–6]. However, whereas these studies have provided considerable information about cellular characteristics in CTCL, the hope that they would aid in the early diagnosis has not been fulfilled [1–6].

Gene mapping with the use of DNA probes specific for immunoglobulin (lg) heavy and light chain genes has provided a useful supplement to establish clonality in B-cell tumours [7,8]. Recently, cloning of the genes that encode the beta-chain of the antigen specific T-cell receptor (TCR) has provided a comparable technique to document clonality in T-cell proliferations [9–11]. These genes have an organization similar to that of lg genes (Fig 1), undergo rearrangement early in T-cell development, [12,13] and have been shown to be rearranged in a clonal fashion in most T-cell leukemias and lymphomas [14–18], including skin and lymph node biopsy specimens from advanced (tumor) stages of CTCL [19]. However, the value of such DNA analysis in the early diagnosis of CTCL remains unclear.

In the present study, the configuration of the TCR beta-chain gene has been investigated in skin, blood, and lymph node samples from known or suspected CTCL in an attempt to establish the usefulness of this novel technique in the early diagnosis of CTCL.

MATERIAL AND METHODS

Patients  Twenty-three patients (17 men and 6 women) followed in the Department of Dermatology at the Finsen Institute for periods of between 1–11 years (median, 4 years) were selected for study. Their ages ranged from 15–84 years (median, 67 years). The diagnoses are summarized in Table I and were based on standard clinical [20,21] and histologic [22,23] criteria, all available histopathologic specimens being reevaluated by one of the authors (ER).

All cases classified as mycosis fungoides (including stage II, plaque lesions [see Table I]) showed epidermotropic lymphoid infiltrates with classical Pautrier microabscesses and many pleomorphic lymphoid cells with large, hyperchromatic and irregular nuclei [23]. In the suspected plaque or erythrodermic lesions (cases 1–8, see Table I), pleomorphic lymphocytes were more infrequent. Furthermore, although in these lesions single cell exocytosis was usually prominent, Pautrier microabscesses could not be identified in routinely processed serial sections [24].

Clinical Evaluation and Staging Systems  The clinical evaluation included physical examination, bone marrow examination, liver biopsy, lymphangiography, or lymph node biopsy [21].

For mycosis fungoides (MF), the staging system proposed by the Scandinavian Mycosis Fungoides Cooperative Group [21] was adopted: stage I = plaque lesions of MF, no extracutaneous disease; stage III = tumor lesions of MF, no extracutaneous disease; stage IV = MF with enlargement of lymph nodes, showing...
either dermatopathic lymphadenitis (stage IVa) or malignant lymphoma (stage IVb) histologically.

Patients with cutaneous large cell non-Hodgkin’s lymphomas were staged in accordance with the Ann Arbor system [25].

**Cells and Tissue Samples** Fresh unfixed biopsy specimens and Lymphoprep® isolated blood mononuclear cells were frozen and stored in liquid nitrogen. Skin specimens were obtained as 4 mm punch biopsies. From individual patients, 2–13 skin biopsies were available for the gene mapping analysis (Table I). In 5 cases (nos. 4, 8, 12, 17, and 22, Table I), skin biopsies were obtained during observation periods of 1–7 months and were pooled prior to DNA analysis. In these cases, discase was stable during the observation period both clinically and histologically. In the remaining cases, skin biopsy specimens were obtained by taking multiple biopsies from individual lesions.

All specimens were examined morphologically (to assure representative sampling) and immunocytochemically, using standard immunoperoxidase [26] or immunoealkaline phosphatase [27] staining methods and a panel of monoclonal antibodies against lymphoid cells described previously [5]. In 21 cases, the infiltrating cells expressed a helper/inducer (CD4-positive, CD8-negative) phenotype. In the remaining 2 cases, the neoplastic cells showed unusual phenotypes in that they either co-expressed the antigens CD4 and CD8 (case no. 15) or lacked reactivity with both markers (case no. 23, Table I).

Controls consisted of samples of tissue from 28 cases of carcinoma and peripheral blood from 36 healthy white volunteers.

**DNA Analysis** DNA was extracted from frozen tissue biopsy specimens (or peripheral blood leukocytes) by routine methods and digested with endonucleases [28]. The DNA fragments were subjected to electrophoresis in 0.8% agarose gels and transferred to nitrocellulose filters by Southern blotting. Filters were hybridized with 32P-labeled probes specific for Ig and TCR genes [29], washed under appropriate conditions, and subjected to autoradiography. The 4 probes used in this study were an Ig heavy-chain joining region JH probe (C76R51A), an Ig kappa-chain constant region Ck probe (pUCR17Ck), an Ig lambda-chain constant human C locus probe (pUCR17Ck), and an Ig kappa-chain constant

### Table I. Clinical, Immunophenotypic, and Genotypic Findings in Cutaneous T-cell Lymphomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Samples Studied</th>
<th>Phenotype</th>
<th>Gene Configuration</th>
<th>Ig Heavy Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>? Early CTCL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>2</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>3</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>4</td>
<td>Skin (7)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>5</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>6</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>7</td>
<td>Skin (4)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>8</td>
<td>Skin (13)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>MF plaque, stage II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Skin (4)</td>
<td>+</td>
<td>+</td>
<td>GNN</td>
</tr>
<tr>
<td>10</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>11</td>
<td>Skin (3)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>12</td>
<td>Skin (5)</td>
<td>+</td>
<td>+</td>
<td>GGG</td>
</tr>
<tr>
<td>MF tumor, stages III and IVa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Skin (5)</td>
<td>+</td>
<td>+</td>
<td>NRR</td>
</tr>
<tr>
<td>14</td>
<td>Skin (2)</td>
<td>+</td>
<td>+</td>
<td>RGR</td>
</tr>
<tr>
<td>15</td>
<td>Lymph node</td>
<td>+</td>
<td>+</td>
<td>RGR</td>
</tr>
<tr>
<td>16</td>
<td>Lymph node</td>
<td>+</td>
<td>+</td>
<td>RRR</td>
</tr>
<tr>
<td>17</td>
<td>Skin (5)</td>
<td>+</td>
<td>+</td>
<td>RRR</td>
</tr>
<tr>
<td>Sézary syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Blood</td>
<td>+</td>
<td>+</td>
<td>RRN</td>
</tr>
<tr>
<td>19</td>
<td>Blood</td>
<td>+</td>
<td>+</td>
<td>RRN</td>
</tr>
<tr>
<td>20</td>
<td>Blood</td>
<td>+</td>
<td>+</td>
<td>RRN</td>
</tr>
<tr>
<td>21</td>
<td>Blood</td>
<td>+</td>
<td>+</td>
<td>RRN</td>
</tr>
<tr>
<td>Lymphoblastic or immunoblastic lymphoma, stage Ie</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Skin (5)</td>
<td>+</td>
<td>+</td>
<td>RRR</td>
</tr>
<tr>
<td>23</td>
<td>Lymph node</td>
<td>+</td>
<td>+</td>
<td>RRR</td>
</tr>
<tr>
<td>24</td>
<td>Skin (2)</td>
<td>+</td>
<td>+</td>
<td>NRR</td>
</tr>
</tbody>
</table>

2 Early CTCL = clinically suspicious, histologically nondiagnostic poikiloderma plaques (nos. 1–6) or erythrodermic lesions (nos. 7, 8); MF = mycosis fungoides.

2Numbers in parentheses indicate the number of skin biopsies available for the gene mapping analysis in individual patients. Lymph node biopsy specimens from cases 15, 16, and 22 showed dermatopathic lymphadenitis histologically.

2For Ig genes results were obtained with more than one enzyme in 19 of 22 cases. For TCR beta-chain genes, results are expressed after digestion with BamHI, EcoRI and HindIII, respectively. R = rearranged; G = germline (nonrearranged) configuration; and N = not analyzed.
region C probe (Chr 22 lambda 15) [30], and a TCR beta-chain gene probe (Jurkat beta 2) [11].

**Immunoglobin Genes:** Aliquots of all DNA samples were digested with BamHI and either EcoRI or HindIII before hybridization with the Jα probe. DNA was digested with BamHI prior to Cα and HindIII before Cα probe hybridization. All control samples showed identical restriction fragment patterns with the above enzymes and probes; no DNA polymorphisms or rearrangements were observed.

**T-Cell Receptor Beta-Chain Gene:** Aliquots of all DNA samples were digested with BamHI, EcoRI, and HindIII before hybridization with the TCR beta-chain gene probe. Dilution experiments have shown that in our hands, this technique can detect a T-cell clone at a 5% level [17]. All control samples showed identical patterns with all enzymes.

A rearrangement was defined as having occurred if after digestion with restriction endonucleases a new band was detected on hybridization with 1 gene probe. Where possible, all rearrangements were shown using at least 2 different restriction enzymes, in order to exclude the possibility of abnormal patterns being caused by DNA polymorphism.

**RESULTS**

The results from the study of TCR and Ig genes in skin, blood, and lymph node specimens from 23 patients with a proven or suspected diagnosis of CTCL are summarized in Table I.

The TCR beta-chain gene showed a germline (nonrearranged) configuration in all specimens from 8 patients with clinically suspicious, but histologically nondiagnostic poikilodermic plaques (nos. 1–6, Table I) or erythrodermic lesions (nos. 7–8, Table I). A germline pattern was also found in all samples from 4 patients (nos. 9–12, Table I) with histologically verified cutaneous plaque stages of MF (Fig 2).

This was in clear distinction to aggressive (i.e., immunoblastic or lymphoblastic) variants of CTCL (nos. 22 and 23, Table I) or advanced stages of CTCL (i.e., Sézary syndrome, tumor stages of MF—nos. 13–21, Table I) in which the TCR beta-chain gene showed a clonal rearrangement pattern in all samples (Fig 2), including 3 lymph nodes biopsies (nos. 15, 16, and 22, Table I) in which histologic examination had only revealed dermatopathic lymphadenitis.

No 2 cases of CTCL showed a similar rearrangement pattern, but in case 15 and 22 the clonal TCR beta-chain rearrangement patterns were identical from skin and lymph node tissue.

Ig heavy- and light-chain genes were present in a germline configuration in all cases with the exception of case 19 (Sézary syndrome) in which a clonal rearrangement pattern was found for Ig heavy-chain genes.

**DISCUSSION**

It is generally accepted that the great majority of malignant lymphomas represent a monoclonal proliferation of either B- or T-lymphocytes. Whereas the demonstration of Ig light-chain restriction [26,31] or clonal rearrangement of Ig genes [7,8] has provided a valuable means of documenting monoclonality in B-cell lymphomas, no comparable technique has been available for the objective demonstration of clonality in T-cell neoplasms.

Recently, the availability of gene probes for the beta-chain of the TCR [11] has provided a means of demonstrating monoclonality in T-cell proliferations by showing rearrangements of this gene in clonal fashion in DNA extracted from blood or tissue samples (Fig 1). Several recent reports have used this technique to document T-cell lineage and clonality in leukemias and lymphomas [14–18] and advanced (tumor) stages of CTCL [19], but no previous studies have focused on the value of this method in the diagnosis of the early stages of CTCL.

In the present study the configuration of the TCR beta-chain gene has been investigated in skin, blood, and lymph node samples from various types and stages of CTCL in an attempt to establish whether this method is useful in the early recognition of CTCL. The results indicate that aggressive variants of CTCL (i.e., lymphoblastic or immunoblastic lymphomas) and advanced stages of CTCL (i.e., Sézary syndrome, tumor stages of MF) all show clonal rearrangements of the TCR beta-chain gene (including histologically nondiagnostic lymph node lesions). This finding is in keeping with a previous study [19] and supports the view that in these stages, the disease is clonal [32] and shows a high frequency of dissemination to extracutaneous sites [33,34].

In contrast, the lesions of plaque stages of mycosis fungoides and early suspected CTCL consistently possessed the TCR beta-chain gene in a germline configuration. This may be explained by the fact that the infiltrating T-cells in these lesions are polyclonal rather than monoclonal (i.e., they are nonneoplastic). An alternative possibility is that in these lesions, abnormal T-cell clones are present at a level which is too low to be detected, i.e., they constitute (if present) less than 5% of the cells studied. Hence, in dilution experiments, we and others have shown that with the use of DNA probes specific for the TCR beta-chain gene a T-cell clone can be detected at levels down to 5% [17,18]. Whatever explanation is correct, these findings may help to explain the indolent course and favorable prognosis seen in most early cases of CTCL [21,35]. They also offer an explanation for the close immunophenotypic and cell kinetic similarity between these conditions and benign cutaneous T-cell infiltrates [3–6].

![Figure 2](image-url)
In conclusion, this investigation indicates that DNA analysis of CTCL with TCR beta-chain gene probes forms a useful supplementary technique in documenting extracutaneous disease in advanced CTCL. In the early stages there was no evidence of clonality, suggesting that in these lesions the T-cell proliferation is either polyclonal or contains only very few monoclonal (i.e., neoplastic) cells.

The authors are grateful to Professor David Weatherall and Dr. Kristian Thomsen for support and encouragement, Dr. Tak Mak for the T-cell receptor probe, and Dr. Terence Rabbits for the Ig gene probes.

REFERENCES


T-CELL RECEPTOR GENES IN CUTANEOUS LYMPHOMAS