CONCISE REPORT

Analysis of T-Cell Receptor Beta Chain (Tβ) Gene Rearrangements Demonstrates the Monoclonal Nature of T-Cell Chronic Lymphoproliferative Disorders

By Robin Foa, Pier-Giuseppe Pelicci, Nicola Migone, Francesco Lauria, Giovanni Pizzolo, Frances Flug, Daniel M. Knowles II, and Riccardo Dalla-Favera

We investigated the rearrangement patterns of the gene coding for the beta chain of the T cell receptor (Tβ) in 11 patients with T-cell derived chronic lymphoproliferative disorders, including T-cell prolymphocytic leukemia (T-PLL) and T-cell chronic lymphocytic leukemia (T-CLL). We found that all five cases of T-PLL and five of six cases of T-CLL displayed Tβ-gene rearrangements, clearly establishing their monoclonal nature. Clonality could not be determined in one case of T-CLL where the Tβ gene was found unrearranged. Our results demonstrate that the majority of cases of both clinically aggressive T-PLL and clinically indolent T-CLL are monoclonal. These results suggest that the analysis of Tβ gene rearrangements represents a valid tool for the differential diagnosis and clinical monitoring of T-cell derived chronic lymphoproliferative diseases.

T-CELL chronic lymphoproliferative disorders are well-recognized clinicopathologic diseases, which include T-cell prolymphocytic leukemia (T-PLL) and T-cell chronic lymphocytic leukemia (T-CLL). T-PLL is usually characterized by clinical features suggesting a malignant process, ie, a high WBC count, marked splenomegaly, an aggressive clinical course and a limited therapeutic response. T-CLL, including large granular lymphocyte (LGL) proliferations (T-,lymphoproliferative disease, T-LPD), are generally characterized by a relatively low WBC count, an indolent, nonprogressive clinical course and prolonged survival. The benign clinical course of many cases of T-CLL has led some authors to question their neoplastic nature and to recommend the term chronic T-cell lymphocytosis unless disease progression is demonstrable. Difficulty in determining the neoplastic or the reactive nature of these cases has stemmed from the lack of an adequate marker of clonality for T-cell populations.

Recently, DNA sequences coding for the beta chain of the T-cell receptor (Tβ) have been isolated. It has been shown that the Tβ gene undergoes structural rearrangements that precede and are necessary for Tβ gene expression in T-cells, analogous to immunoglobulin gene rearrangements in B-cells. We and others have demonstrated that the patterns of Tβ gene rearrangement, identified by Southern blot hybridization analysis using Tβ gene probes, can be used to identify the monoclonality of a given T-cell population. We used this immunogenotypic approach, in the studies described here, to demonstrate that the vast majority of T-cell chronic lymphoproliferative disorders, including clinically aggressive T-PLL and clinically indolent T-CLL, are monoclonal T-cell proliferations.

PATIENTS AND METHODS

Patients and diagnosis. We studied six cases of T-CLL and five cases of T-PLL. Diagnoses were established by conventional clinical, morphological, and phenotypic criteria. All six patients with T-CLL exhibited a benign clinical course and a moderate lymphocytosis. The cells in five patients (Table 1, cases 2 through 6) displayed typical LGL morphology, ie, abundant azurophilic granular rich cytoplasm, and absent nucleoli. The cells in one patient (Table 1, case 1) were small, mature appearing lymphocytes, without nucleoli and without LGL morphology. All five patients with T-PLL exhibited an aggressive clinical course and a lymphocytosis of intermediate sized cells with moderately basophilic cytoplasm, vesicular nuclei, and prominent nucleoli.

Immunophenotypic analysis. Sheep erythrocyte (E) rosette formation was assayed with Vibrio Cholera neuraminidase-treated sheep erythrocytes. T-cell associated antigen expression was determined by indirect immunofluorescence using murine monoclonal antibodies OKT3, OKT4, OKT6, OKT8, OKT11, and Leu7. Cytotoxicity assays. NK activity was measured using K562 leukemia cells as target cells, at effector to target cell (E:T) ratios ranging from 25:1 to 100:1, in a four hour 14Cr release assay. For ADCC, 11Cr-labelled TLX9 lymphoma cells, sensitized with rabbit antibody, were used as targets in a four hour test.

Gene rearrangement studies. DNA purification was performed by cell lysis, proteinase K digestion, extraction with phenol/chloroform and precipitation with ethanol. Fifteen micrograms of DNA extracted from each sample were digested with the appropriate restriction endonuclease, electrophoresed in a 0.8% agarose gel, and purified by electrophoresis in a 0.8% agarose gel. Gene rearrangement studies. DNA purification was performed by cell lysis, proteinase K digestion, extraction with phenol/chloroform and precipitation with ethanol. Fifteen micrograms of DNA extracted from each sample were digested with the appropriate restriction endonuclease, electrophoresed in a 0.8% agarose gel, and purified. 

Clinica Medica A, Istituto di Ematologia and Centro Nazionale Ricerche (CNR) Immuongenetika ed Istocompatibilita, University of Torino, Torino, Italy; Istituto di Ematologia "L.A. Seragnoli," University of Bologna, Bologna, Italy; Cattedra di Ematologia, University of Verona, Verona, Italy; Department of Pathology, Pediatrics and Kaplan Cancer Center, New York University School of Medicine, New York.

Supported in part by C.N.R., Progetto Finalizzato, Oncologia Grant No. 84.00581.44 (R.F.), National Institutes of Health Grants No. CA47165, CA47295 (R.F.) and EY03335 (D.M.K.). P.G.P. is supported by a Fellowship from the Italian-American Association for Cancer Research. D.M.K. is supported in part by the Cancer Research Institute, the New York State AIDS Institute (A0122), and by the Bernard and Frances Laterman Project Chai Philanthropic Trust. R.D.F. is a Scholar of the Leukemia Society of America.

Submitted July 22, 1985; accepted Sept 9, 1985.

Address reprint requests to Riccardo Dalla-Favera, MD, Department of Pathology, New York University School of Medicine, 550 First Ave, Rm 544, MSB, New York, NY 10016.

© 1986 by Grune & Stratton, Inc.
RESULTS

In general, these 11 cases displayed the typical clinical and phenotypic characteristics of T-CLL or T-PLL (Table 1). In some cases, the cells displayed a relatively infrequent phenotype, the simultaneous expression of T4 and T8. This phenotype is exceptional in T-CLL and infrequent in T-PLL.16

We previously demonstrated that the analysis of T, gene rearrangements by Southern blot hybridization using T, probes, allows the definition of the lineage, ie, T-cell, and the monoclonal nature of a given lymphoid cell population.12 Figure 1 (lanes A) illustrates the pattern of hybridization bands obtained when human DNA from a non-T-cell population is digested with EcoRI or BamHI and hybridized to the T, probe. The same analysis, performed on DNA obtained from a polyclonal T-cell population, represented by normal human peripheral blood T-lymphocytes, shows the biallelic deletion of the 12.0 kb band upon digestion with EcoRI and a conserved germline pattern upon digestion with BamHI (Fig 1, lane B). In monoclonal T-cell populations, the restriction pattern of the gene is characterized by the same EcoRI band deletion and in addition, by the presence of one or more rearranged band(s), which can be detected by digestion with at least one restriction enzyme (see ref. 12 and below).

We analyzed the six cases of T-CLL and five cases of T-PLL based upon this scheme. Southern blot analysis demonstrated the presence of T, gene rearrangements in all five cases of T-PLL and in five of six cases of T-CLL (Table 1). All of the cases but one displayed the disappearance of the 12.0 kb EcoRI band and the appearance of new hybridization bands upon DNA digestion with EcoRI and/or BamHI (or HindIII, not shown) restriction enzymes. Three cases displaying T, gene rearrangements are illustrated in Fig 1. Cases 3 and 11 showed the appearance of rearranged bands upon DNA digestion with both EcoRI and BamHI. In Case 3, the absence of the EcoRI and BamHI germline fragments together with the appearance of a single rearranged band, suggests the deletion of one allele of the T, gene and the rearrangement of the other. A rearranged band in Case 10 is apparent only upon DNA digestion with BamHI restriction enzyme. The single case without rearranged bands also showed conservation of the EcoRI 12.0 kb band (see 6 in Fig 1), indicating that the T, gene is unrearranged in these cells. This finding was confirmed by digestion of the same DNA with BamHI (Fig 1, Lane 6) and HindIII (not shown) restriction enzymes. Analysis of the immunoglobulin heavy chain locus showed a germline configuration in all 11

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>T, Gene*</th>
<th>E/11</th>
<th>T3</th>
<th>T4</th>
<th>T6</th>
<th>T8</th>
<th>Leu7</th>
<th>NK</th>
<th>ADCC</th>
<th>Total Lymphocytes</th>
<th>Spleen</th>
<th>Liver</th>
<th>Lymph Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T-CLL</td>
<td>R</td>
<td>+ /NT</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>17.2</td>
<td>75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>T-CLL</td>
<td>R</td>
<td>+ /NT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>7.4</td>
<td>8.2</td>
<td>65</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>T-CLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>25.1</td>
<td>70</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>T-CLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>9.1</td>
<td>73</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>T-CLL</td>
<td>R</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.2</td>
<td>78</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>T-CLL</td>
<td>G</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>59</td>
<td>84</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>T-PLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>79</td>
<td>92</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>T-PLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>120</td>
<td>90</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>T-PLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>259</td>
<td>99</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>T-PLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>49</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>T-PLL</td>
<td>R</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* R rearranged; G, germline
† NT, not tested.
‡ WBC, white blood cell count expressed as WBC number × 10³/µL.
cases (data not shown). Thus, our data indicate that ten of the 11 T-cell chronic lymphoproliferative disorders are monoclonal T-cell proliferations, since the cells display clonal \( T_\alpha \) gene rearrangements. Clonality could not be determined by this approach in one case of T-CLL, since the \( T_\beta \) gene was found to be unarranged.

**DISCUSSION**

This study sought to determine the mono- or the poly-clonality of T-cell chronic lymphoproliferative disorders, including T-PLL and T-CLL. In contrast with most other neoplasms, the monoclonality of some of these cases, particularly T-CLL, cannot be assumed or easily established in view of: (1) their nonaggressive, clinical behavior; (2) the lack of cytogenetic markers to confirm both their clonality and, by analogy with other neoplasms, their malignancy; and (3) the lack of clearly identifiable morphologic or immunophenotypic features to distinguish them from their normal, benign counterparts.

The studies presented here demonstrate that the vast majority of T-cell chronic lymphoproliferative disorders are monoclonal. The monoclonal and probable malignant nature of T-PLL is strongly suggested by its typical aggressive clinical course. However, the monoclonal nature of T-CLL was also established in five of six cases. The latter cases display a nonaggressive clinical course and immunophenotypic features, which pose reasonable doubts in terms of the differential diagnosis between a benign chronic lymphocytosis and a true lymphoid malignancy. For instance, Case 2 displayed a particularly benign clinical course during three years of follow-up. We also demonstrated that some chronic lymphoid proliferations which display a mixed T4/T8 phenotype are monoclonal. These cases may be more frequent than previously reported, and may have often been inappropriately classified as chronic T-cell lymphocytoses by immunophenotypic analysis.

One case of T-CLL exhibited an unarranged \( T_\beta \) gene configuration. The cells from this case exhibited LGL morphology and NK function, although their T3- T4- T8- phenotype (Table 1) differed from the other cases of T-CLL displaying LGL features. These observations suggest that LGL-derived T-CLLs are immunogenotypically and immunophenotypically heterogeneous. They may be derived from more than one lineage and/or different stages of differentiation. This heterogeneity may reflect the heterogeneity of the normal LGL population and is consistent with our preliminary observations that \( T_\beta \) gene rearrangement and expression is found in only a subpopulation of normal peripheral blood LGL cells (Pelicci PG et al, manuscript in preparation). With respect to the present study, we conclude that the large category of T-CLL may include a distinct subset of cases with LGL morphology, the monoclonal nature of which remains to be established.

Establishing the monoclonal nature of these cases bears important implications for their differential diagnosis, prognosis, and therapeutic management. First, it is important to note that the monoclonality of these diseases does not necessarily imply their complete malignancy. At least some of these cases may represent early, yet persisting stages of a multi-step leukemogenic process. The existence of cases that undergo a progressive change in their clinical course switching toward a more aggressive and malignant phenotype supports this notion. Analysis of \( T_\beta \) gene rearrangements in longitudinal studies is likely to be useful in determining the derivation of these more malignant clones, by comparing their genotype and phenotype with those cells of earlier stages of the disease. The determination of clonality by \( T_\beta \) gene rearrangement analysis may be of significant diagnostic and prognostic value. Although our study suggests that even the most clinically chronic T-cell proliferations are of monoclonal origin, immunogenotypic analysis may still be needed to clarify difficult diagnostic situations. Once the determination of monoclonalities has been made, the immunogenotypic marker would appear to represent a convenient tool with which to follow the biologic behavior and the therapeutic response of the pathologic clone, thus allowing improved monitoring of the patients' clinical course.

**ACKNOWLEDGEMENT**

We thank Dr Tak Mak for providing the \( T_\beta \) probe, Michel Brathwaite and Angela Ubriaco for excellent technical assistance, and Diane Nazario and Margaret Walden for preparing the manuscript.

**REFERENCES**

Analysis of T-cell receptor beta chain (T beta) gene rearrangements demonstrates the monoclonal nature of T-cell chronic lymphoproliferative disorders

R Foa, PG Pelicci, N Migone, F Lauria, G Pizzolo, F Flug, DM 2d Knowles and R Dalla-Favera