T Cell Receptor Gene Rearrangements Define a Monoclonal T Cell Proliferation in Patients With T Cell Lymphocytosis and Cytopenia


We have used probes from the T cell receptor β and γ chain loci to investigate the clonality of T lymphocytes in eight patients with T cell lymphocytosis and cytopenia (TCLC). This syndrome, which is strongly associated with rheumatoid arthritis, is characterized by peripheral blood and bone marrow lymphocytosis and neutropenia, red cell aplasia, or both. By means of T cell monoclonal antibodies and flow cytometry, T lymphocytes from patients with this syndrome have been shown to have characteristic immunologic features. Investigators have disagreed as to whether the syndrome represents a T cell malignancy or a more benign immunologic disorder. DNA from five of five patients with symptomatic “classic” T cell lymphocytosis with cytopenia demonstrated unique rearrangements of the T cell receptor β chain locus, whereas neither of two patients with atypical features showed rearrangement. In addition, we found evidence for γ chain rearrangement in those DNAs with clonal β chain rearrangement. We thus postulate that the classic form of this syndrome is associated with a monoclonal proliferation of T cells. Its potential relationship to T cell chronic lymphocytic leukemia is discussed.

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THE SYNDROME of T cell lymphocytosis associated with granulocyte or erythrocyte aplasia is a rare but distinct clinical entity.1-4 Patients initially may have recurrent fever and infections associated with neutropenia or symptoms of fatigue related to anemia. Diagnosis is sometimes made by routine blood testing in asymptomatic patients. There is a strong association of this syndrome with rheumatoid arthritis (RA); in the largest reported series of these patients, one third had RA.5 Physical findings include splenomegaly, but hepatomegaly or lymph node enlargement is rare. Most patients have neutropenia, a few patients have erythrocyte aplasia, and rare patients have both. Thrombocytopenia is unusual. All patients have a peripheral blood lymphocytosis with an associated infiltration of their bone marrow with mature-appearing lymphocytes. In neutropenia, the marrow usually shows a predominance of early myeloid forms; in red cell aplasia, there may be a complete absence of erythrocyte precursors in the marrow. The course of the disease in most patients is chronic and only the minority require treatment, although some patients have died from their disease.

Previous studies have used T cell functional studies as well as monoclonal antibodies and flow cytometry to characterize the peripheral blood lymphocytes in these patients.5-4 The T cells display a characteristic phenotype. They are CD8 (Leu2,OKT8) positive, characteristic of suppressor T cells, but they are negative, or only weakly positive, for a panel of pan-T cell CD5 markers. They also usually express the Leu7 and H366 antigens and receptors for the Fc portion of IgG, markers typically associated with natural killer cell activity.

The uniformity of the expression of these characteristic T cell antigens in the setting of the lymphocytic infiltration of the bone marrow and peripheral blood has led many investigators to postulate that this represents a monoclonal disorder related to chronic lymphocytic leukemia.9 However, the indolent clinical course of the disease in most patients and its strong association with rheumatoid arthritis have suggested to others a more benign immunologic disorder.7 Many of these patients have been given a diagnosis of Felty’s syndrome. However, these patients can be distinguished from patients with “classic” Felty’s syndrome by surface marker studies of peripheral T cells; patients undergoing splenectomy can be distinguished from those with Felty’s syndrome by histologic examination of their spleens.4

The availability of probes for T cell receptor genes offers an opportunity to help resolve this controversy. The genes for the β chain of the T cell receptor have been cloned and have been shown to rearrange in a manner analogous to the immunoglobulin genes in B lymphocytes.10 The immunoglobulin genes are encoded on discontinuous genes. During the course of B cell differentiation, these genes undergo rearrangement to form functional continuous genes which encode for antibody. These rearrangements are detectable by DNA blotting techniques, and rearrangement studies have been used to establish monoclonality of B cell tumors,11 to diagnose malignancy in the face of confusing histology,12 and to show that some classic lymphomas are in fact biclonal.13 We have used the rearrangement of the β chain genes of the T cell receptor as a marker for monoclonality of T cells in an analogous manner. We have performed Southern blot analysis of the T cell receptor β chain genes in six patients with classic T cell lymphocytosis and cytopenia (TCLC) and in two patients with an atypical presentation. We have demonstrated unique rearrangements of the β chain genes in five of five patients symptomatic with the classic syndrome. We did not find rearrangements in a sixth patient from whom only remission blood was available or in two patients with atypical features.

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T CELL PROLIFERATION IN PATIENTS WITH TCLC

Detection of T cell receptor β chain rearrangement. The organization of the β chain locus is diagrammed in Fig 1. The two constant regions (Cβ1 and Cβ2) are physically linked and lie approximately 8 kilobases (kb) from one another. The probe used to analyze genomic DNA was a 1.0 kb Bgl II to Bgl II fragment from Cβ2. The two constant region genes are highly homologous in the coding region; therefore, this fragment hybridizes equally to both genes as demonstrated in Fig 1. On digestion of DNA with Eco RI and hybridization to the probe, two restriction fragments are predicted: a 10.8-kb fragment resulting from hybridization to Cβ1, and a 3.7-kb fragment resulting from hybridization to Cβ2. Because there is an Eco RI site 3' to the Jβ2 genes, this probe will only detect rearrangements involving the joining (J) region of Cβ1. Rearrangements involving the Cβ2 genes can be detected on hybridization of the same probe to Hind III digests of genomic DNA. This digest results in three germ-line bands of 7.5, 6.5, and 3.4 kb. The first band represents hybridization to a fragment which includes the J region of Cβ2, and allows detection of rearrangements into that locus. We detected unique rearrangements of the T cell receptor β chain in DNA from all five symptomatic patients with

Table 1. Clinical Characteristics of Patients with TCLC

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Initial Complaint</th>
<th>H.O RA</th>
<th>Affected Cell Line</th>
<th>Treatment</th>
<th>Survival (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>46</td>
<td>Routine</td>
<td>+</td>
<td>Eryth</td>
<td>Spl</td>
<td>&gt;10</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>68</td>
<td>FUO</td>
<td>+</td>
<td>Neut</td>
<td>Chl, Pred</td>
<td>&gt;1</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>64</td>
<td>Fatigue</td>
<td>Se*</td>
<td>Eryth</td>
<td>Pred</td>
<td>3 (LFU)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>48</td>
<td>UTI</td>
<td>+</td>
<td>Neut</td>
<td>Chl</td>
<td>&gt;3</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>55</td>
<td>Abdominal Pain</td>
<td>-</td>
<td>Neut</td>
<td>Chl</td>
<td>&gt;6</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>59</td>
<td>Infection</td>
<td>+</td>
<td>Neut</td>
<td>Spl</td>
<td>&gt;6</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>87</td>
<td>Enteritis</td>
<td>-</td>
<td>Pan</td>
<td>None</td>
<td>&lt;1</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>53</td>
<td>UTI</td>
<td>-</td>
<td>Neut</td>
<td>None</td>
<td>&gt;3</td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; FUO, fever of unknown origin; UTI, urinary tract infection; Eryth, erythrocytes; Neut, neutrophils; Spl, splenectomy; Aza, azathioprine; ChI, chlorambucil; Pred, Prednisone; And, androgens; MCAB, monoclonal antibody; LFU, lost to follow-up.

*Serology only.

The γ chain genes from the mouse and from humans have also been cloned recently. In the mouse, functional somatic rearrangement has been shown to occur primarily in cytotoxic T cells. Our analysis of the DNAs from TCLC patients with β chain rearrangement showed evidence of γ chain rearrangement.

MATERIALS AND METHODS

DNA extractions were performed as described previously. In brief, high mol wt DNA was prepared from spleen cells from patient 1 and from E rosette-purified peripheral blood T cells obtained from the remaining patients. When available, cells used were from patients both when they were symptomatic and when they were in remission. DNA samples from B cell lines from the same patients were used for controls. Cells were incubated overnight at 37 °C in 0.5% sodium dodecyl sulfate (SDS) and 0.2 mg/mL Proteinase K. This was followed by phenol-chloroform extraction and ethanol precipitation. DNA was digested with appropriate restriction enzymes, size-fractionated by agarose gel electrophoresis, and trans-ferred onto nitrocellulose paper by the method of Southern. Filters were hybridized to nick-translated, 32P-labeled probes of the T cell receptor β and γ chain loci and washed at 55 °C in 0.1% SDS/0.15 mol/L of NaCl and 0.0015 mol/L of Na citrate prior to autoradiography.

RESULTS

Table 2. Initial Laboratory Findings in TCLC Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Hb (g/L)</th>
<th>Neut (x 10^9/L)</th>
<th>Lymp (x 10^9/L)</th>
<th>PLT (x 10^9/L)</th>
<th>BML (%)</th>
<th>E (x 10^9/L)</th>
<th>γFcR</th>
<th>CD5</th>
<th>CD4</th>
<th>CD8</th>
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<tbody>
<tr>
<td>1</td>
<td>8.5</td>
<td>1.9</td>
<td>9.0</td>
<td>315</td>
<td>30</td>
<td>85</td>
<td>65*</td>
<td>24*</td>
<td>10*</td>
<td>81*</td>
</tr>
<tr>
<td>2</td>
<td>10.4</td>
<td>0.5</td>
<td>11.8</td>
<td>235</td>
<td>60</td>
<td>69</td>
<td>ND</td>
<td>28*</td>
<td>4*</td>
<td>85*</td>
</tr>
<tr>
<td>3</td>
<td>4.4</td>
<td>2.5</td>
<td>7.9</td>
<td>445</td>
<td>45</td>
<td>88</td>
<td>68*</td>
<td>27*</td>
<td>13*</td>
<td>77*</td>
</tr>
<tr>
<td>4</td>
<td>10.9</td>
<td>0.5</td>
<td>4.0</td>
<td>130</td>
<td>30</td>
<td>78</td>
<td>66</td>
<td>40</td>
<td>ND</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>10.8</td>
<td>0.6</td>
<td>11.5</td>
<td>257</td>
<td>30</td>
<td>83</td>
<td>97*</td>
<td>16*</td>
<td>4*</td>
<td>95*</td>
</tr>
<tr>
<td>6</td>
<td>13.3</td>
<td>0.3</td>
<td>5.5</td>
<td>100</td>
<td>36</td>
<td>87</td>
<td>92*</td>
<td>34*</td>
<td>9*</td>
<td>82*</td>
</tr>
<tr>
<td>7</td>
<td>8.0</td>
<td>0.8</td>
<td>1.9</td>
<td>62†</td>
<td>30</td>
<td>89</td>
<td>ND</td>
<td>56</td>
<td>26</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>12.7</td>
<td>0.5</td>
<td>1.9</td>
<td>140</td>
<td>37</td>
<td>50</td>
<td>ND</td>
<td>45</td>
<td>34</td>
<td>23</td>
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<tr>
<td>NL</td>
<td>12–17</td>
<td>1.8–7.0</td>
<td>1.5–4.0</td>
<td>150–440</td>
<td>&lt;15</td>
<td>65</td>
<td>84%*</td>
<td>44</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Hb, hemoglobin; Neut, neutrophils; Lymp, lymphocytes; PLT, platelets; BML (%), percentage of bone marrow lymphocytes; E*, E rosette positive; γFcR, Fc receptor positive; CD5, OKT1, Leu1; CD4, OKT4, Leu3; CD8, OKT8, Leu2; NL, normal range, ND, not done.

*Value obtained on E rosette-sorted cells.

†Antiplatelet antibodies were present.
was digested with Eco RI or Hind III. The restriction sites for those enzymes are shown.

Fig 1. Organization of the human T cell receptor β chain genes. The two constant region genes (Cβ1 and Cβ2) are shown. Southern blot analysis was performed using a Bgl II-Bgl II probe from Cβ2 which hybridizes equally to both constant regions. DNA was digested with Eco RI or Hind III. The restriction sites for those enzymes are shown.

classic TCLC. Three of these cases are illustrated in Fig 2. In each case, one or more rearranged bands are seen in the DNA from patients at the time of their symptomatic illness; these bands are absent in DNAs from patients in remission (Fig 2; T cells, CR). Case 4, from whom only remission blood was available, revealed no rearrangement. The other five cases all showed rearrangement of Cβ2; only two revealed rearrangement of Cβ1. As discussed below, interpretation of Cβ1 rearrangement was hampered by the presence of a frequent unexplained band in digests of normal DNA.

The analysis of case 1 is unusual in that four rearranged bands are apparent. This is consistent with either the presence of at least two independent clones of T cells or with multiple rearrangements occurring in the same cell. The other cases were definitely consistent with a single clone of abnormal cells because the DNAs revealed only one or two rearranged bands.

Case 6 demonstrates the difficulty of interpreting rearrangements of Cβ1. Although this patient had a clear rearrangement of Cβ2, which in remission disappeared in the expected fashion, we noted the appearance of what appeared to be a single rearrangement of Cβ1 arising de novo in this patient in remission (Fig 3). On further analysis, we noted the frequent appearance of a similar 9-kb band, corresponding to apparent rearrangement of Cβ1, in digests of DNA from patients with unrelated disorders and in DNA from peripheral blood cells of 11 of 11 normal individuals. Minden et al observed an aberrant band in Eco RI digests of genomic DNAs from fibroblasts, which may represent a similar finding. The DNA sequences represented by this band remain to be determined.

Detection of T cell receptor γ chain rearrangement. There are two human γ chain constant region genes. We have shown that mature T cells frequently show deletion of one of the constant region genes that probably occurs in the course of somatic rearrangement of the γ chain genes. DNA from the TCLC patients were analyzed with a probe from the Cγ1 constant region to see if rearrangement of the γ chain genes, with subsequent deletion of the other gene, could be inferred from the loss of one hybridizing band on Southern blot analysis. Partial loss of one band was seen on hybridization of the γ chain probe to DNAs from patients in their symptomatic phase of the illness (Fig 4, T cells); such a deletion is not observed on analysis of DNAs from patients in remission or of DNAs from B cell lines derived from the same patients. One exception is case 1, in which there is still deletion of one of the constant region genes in DNA in remission, but no deletion in B cells. The possible significance of this will be discussed below.

DISCUSSION

The finding of discrete rearrangements of the immunoglobulin genes in B cell proliferative syndromes has been widely viewed as a marker of monoclonality and, by inference, of malignancy. We have applied the same techniques to the examination of a T cell lymphoproliferative disorder and have shown monoclonal rearrangements of the T cell receptor genes. By analogy, we postulate that, in its classic form, TCLC usually represents a clonal expansion of a single or a limited number of T lymphocytes.

Whether this disease should consequently be labeled a "malignancy" is questionable, however. "Benign monoclonal gammopathy" is a syndrome in which a proliferation of a single clone of B cells gives rise to an abnormal circulating immunoglobulin. A small percentage of patients with this syndrome develop multiple myeloma; in the majority, however, it is clinically silent. TCLC may represent a similar disorder of T cells. However, the findings in this syndrome mimic those in B cell chronic lymphocytic leukemia (CLL) in many ways: TCLC is associated with a peripheral blood lymphocytosis, marrow infiltration with lymphocytes, and cytopenia. The finding of monoclonality in this disorder makes it highly analogous to B cell CLL and suggests that TCLC may in fact be a form of T cell CLL.

Two features make TCLC clinically distinct from CLL, however. One is the strong association with RA, a known
autoimmune disease. The other is the observation that the syndrome is not associated with the clinical progression expected with lymphoproliferative diseases in general, and in T cell syndromes in particular. In addition, in some cases, the bone marrow is involved with similar cells, but we have been unable to detect a clonal population of peripheral blood T cells. It may be that in those cases without peripheral lymphocytosis, the clonal population is too small to detect in the peripheral blood. Alternatively, these patients may have a monoclonal population that does not have β chain rearrangement. We have not looked for α chain rearrangement in the DNAs of these patients. Finally, although these cases may simply represent a different entity, they may represent an early stage of the classic disorder from which a clonal disease may emerge. Longitudinal studies and comparisons to similar studies on patients with Felty’s syndrome and patients with other autoimmune diseases may help to clarify this further.

The finding of an aberrant band on analysis of Cβ1 in normal cells is unexplained. The band may represent a true somatic rearrangement, an unexplained cross-hybridizing sequence, or an artifact. Although the significance of this finding in biological terms is not clear, in technical terms, it is extremely important to the correct interpretation of similar studies. This “normal” band may represent a large fraction of total peripheral blood mononuclear cells (in one normal individual, it appeared in equimolar concentration to the germline bands), and may be very misleading in the evaluation of gene rearrangement studies. Normal T cells from the patient, either from remission samples or from phenotypically normal cells sorted from early samples, must be used as controls for Cβ1 rearrangements. We have not noted similar stereotypic rearrangements of Cβ2, and interpretation of rearrangements involving this locus is not difficult.

The suggestion that DNA rearrangement has also occurred in the γ chain locus offers an intriguing avenue for further investigation. Although γ chain rearrangement has been shown to occur at the DNA level in all classes of T cells,15,20 functional rearrangement leading to mRNA transcription has been shown to occur almost exclusively in murine cytolytic T cells.20 This may help to clarify the phenotype of these cells. In vitro studies have demonstrated antibody-dependent cytotoxicity and natural killer activity12 and apparent suppression of marrow growth by subsets of T cells in these patients.6,22 This may suggest that these cells are functional cytotoxic cells that mediate the cellular aplasia characterizing this syndrome, but soluble factor inhibition cannot be excluded. Evidence of functional γ chain production by these cells would suggest that these cells are indeed cytolytic T cells and support the former contention.

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Fig 3. Presence of an aberrant band on Southern analysis of Eco RI digests of normal DNA. Eco RI-digested DNA was analyzed as explained in Fig 1. DNA from patient 6 when in remission (CR) shows a single unique nongermline band which was not present in the asymptomatic (S) phase of the illness. The same band is seen on similar analysis of both lymphocytes and granulocytes from two normal individuals. It is absent in DNA from a B cell line.

Fig 4. Evidence for γ chain rearrangement from Southern blot analysis of DNA from patients with TCLC. DNAs were digested with Hind III, and filters were hybridized to a probe from the Cγ1 locus, yielding two hybridizing bands of 3.5 kb and 2.8 kb in germline DNA. Samples of DNA from each patient during the symptomatic phase of illness show almost total disappearance of the 2.8-kb band, indicating deletion of one of the constant region genes. This is also seen on a remission sample from patient 1, but not on a remission sample from patient 6. DNA from B cell lines from all three patients are shown; in each case, the 2.8-kb hybridizing band is of an intensity equal to that of the 3.5-kb band, indicating a germline configuration without deletion.
Further analysis of the functionality and specificity of the unique T cell receptor gene rearrangements should help to elucidate this further.

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REFERENCES

T cell receptor gene rearrangements define a monoclonal T cell proliferation in patients with T cell lymphocytosis and cytopenia

N Berliner, AD Duby, DC Linch, C Murre, T Quertermous, LJ Knott, T Azin, AC Newland, DL Lewis and MC Galvin