Two distinct types of clinical characteristics and therapies for T-cell large granular lymphocyte leukemia: a report of seven cases

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Abstract: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare, unique type of chronic lymphoproliferative disorder with clinical features including fatigue, anemia, recurrent infection and autoimmune diseases. Here, we report the cases of four T-LGLL patients associated with pure red cell aplasia (PRCA) accompanied by severe anemia and increased lymphocyte ratios. Three T-LGLL patients were characterized by minimal symptoms and indolent disease course for more than three years. Increased lymphocyte ratio and number in peripheral blood, numerous large granular lymphocytes in the peripheral blood and bone marrow, and TCRγ/δ rearrangements were found in these patients. Flow cytometric investigations demonstrated that most T-lymphocytes in the bone marrow were CD3⁺CD8⁺ cells. Identification of clonal expansion of T-lymphocytes by subtyping TCRVβ on T-cells using antibodies against the 24 members of the TCRVβ family is an important method used to ascertain the diagnosis of T-LGLL. Rapamycin is an effective treatment for T-LGLL associated with PRCA without serious side-effects. T-LGLL with minimal symptoms that were untreated was found to remain stable without progression in regular follow-up and laboratory examinations.

Keywords: Large granular lymphocyte, TCRVβ family, pure red cell aplasia, minimal symptoms T-LGLL

Introduction

Increased numbers of lymphocytes in the bone marrow and peripheral blood are frequently seen in a wide range of diseases ranging from mild virus infections to malignancies. Consequently, differential diagnosis is sometimes difficult when the increased lymphocyte numbers last for months or even years and few immature lymphocytes are detected in peripheral blood. Large granular lymphocyte leukemia (LGLL) was first reported by Loughran et al. in 1985 [1], and comprises two types: T-cell LGLL (T-LGLL) and NK-cell LGLL (NK-LGLL), T-LGLL, which is the most common type (75%), is characterized by neutropenia, anemia, PRCA, splenomegaly, rheumatoid arthritis and recurrent bacterial infections. T-LGLL associated with PRCA and minimal symptom T-LGLL is the main types of T-LGLL in Asian countries. The therapy for T-LGLL associated with PRCA is immunosuppressive drugs, primarily using corticosteroids, cyclosporin A, cyclophosphamide and methotrexate [2], although the efficacy is poor in relapsed and refractory patients; therefore, novel therapeutic approaches are urgently required in the clinic. T-LGLL associated with minimal symptom can be misdiagnosed in cases of insidious onset, atypical or mild symptoms without progression for a long period of time, or normal leukocyte counts in peripheral blood. Currently, there is no uniform standard treatment for indolent T-LGLL [3]. Here we report seven cases of T-LGLL with unusual presentation, as well as the diagnostic methods and use of new therapies that differ from the routine strategies.

Case presentation

Between 2013 and 2015, seven patients diagnosed with T-LGLL at the Department of...
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Table 1. Clinical and experimental features of seven patients with T-LGLL

<table>
<thead>
<tr>
<th>Case no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
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<tr>
<td>Age (year)</td>
<td>51</td>
<td>42</td>
<td>61</td>
<td>75</td>
<td>47</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>Main clinical manifestations</td>
<td>Fatigue, facial pallor, ratio of lymphocyte increase</td>
<td>Palpitations, fatigue, ratio of lymphocyte increase</td>
<td>Chronic anemia, palpitations, fatigue</td>
<td>Chronic anemia, ratio of lymphocyte increase</td>
<td>Ratio of lymphocyte increase</td>
<td>Ratio of lymphocyte increase</td>
<td>Ratio of lymphocyte increase</td>
</tr>
<tr>
<td>WBC (×10⁹/L)</td>
<td>7.2</td>
<td>5.8</td>
<td>6.3</td>
<td>7.2</td>
<td>5.3</td>
<td>8.1</td>
<td>7.3</td>
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<tr>
<td>lymphocyte ratio (%)</td>
<td>67</td>
<td>70</td>
<td>78</td>
<td>65</td>
<td>76</td>
<td>63</td>
<td>52</td>
</tr>
<tr>
<td>RBC (×10¹²/L)</td>
<td>1.1</td>
<td>1.8</td>
<td>2.1</td>
<td>2.5</td>
<td>4.3</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>HGB (g/L)</td>
<td>41</td>
<td>63</td>
<td>57</td>
<td>79</td>
<td>132</td>
<td>137</td>
<td>146</td>
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<tr>
<td>Reticulocyte number (×10⁹/L)</td>
<td>5</td>
<td>8</td>
<td>5.2</td>
<td>42</td>
<td>86</td>
<td>81</td>
<td>79</td>
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<tr>
<td>Clonal expansion of TCRVβ and the ratio</td>
<td>TCRVβ5.1 (65.32%)</td>
<td>TCRVβ20 (6.5%)</td>
<td>TCRVβ13.6 (7.52%)</td>
<td>TCRVβ13.6 (13.08%)</td>
<td>TCRVβ14 (38.8%)</td>
<td>TCRVβ1 (36.3%)</td>
<td>TCRVβ22 (30.14%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>T-LGLL, PRCA</td>
<td>T-LGLL, RA</td>
<td>T-LGLL, CA</td>
<td>T-LGLL, CA</td>
<td>T-LGLL</td>
<td>T-LGLL</td>
<td>T-LGLL</td>
</tr>
</tbody>
</table>

RA: refractory anemia; CA: chronic anemia.
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Hematology, Peking University Fist Hospital (China) were enrolled in this retrospective study. The data collected included patient age, sex, the main clinical manifestations, routine blood tests, morphology of cells in blood and bone marrow, TCRVβ family subtyping of bone marrow cells, diagnosis (Table 1), treatment regimen and outcome.

This study was approved by the institutional review board of Peking University Fist Hospital. All patients provided written informed consent.

**Pure red cell aplasia as the main symptom of T-LGLL**

We treated four T-LGLL cases (2 males and 2 females, median age 57 years) with the chief complaint of pallor, weakness and severe anemia. Peripheral blood examinations showed higher lymphocyte ratios (65%-80%), lower reticulocyte ratios (<0.001%) and numbers (<15×10⁹/L), lower hemoglobin (Hb) levels (34-80 g/L) and red blood cell (RBC) counts (<2.6×10¹²/L), while white blood cell (WBC) and platelet (PLT) counts were normal. Physical examinations showed moderate splenomegaly and normal liver size. Granulocyte dysplasia, RBC aplasia, and LGL hyperplasia were identified in the bone marrow in two cases. In the other two cases, LGLs containing variable sized granules were distributed unevenly in abundant cytoplasm and were difficult to identify (Figure 1). Flow cytometric analysis of bone marrow cells from the four cases revealed increased numbers of CD3⁺CD8⁺ and CD5⁺ cells, and decreased numbers of CD19⁺ cells. The TCRαβ T-cell receptor was detected on most T-cells, which were further analyzed using the 24 antibodies for specific detection of the 24 members of the TCRVβ family. Monoclonal expansion of TCRβ5.1, TCRβ20, TCRβ13.6 and TCRβ13.6 was detected in cases 1, 2, 3, and 4, respectively (Figure 2C-F). The four cases were then diagnosed as T-LGLL associated with PRCA based on the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2008 [4].

Rapamycin was the main medication administered for the treatment of these cases. As an example of treatment, in case 1, RBC (3.2×10¹²/L), Hb (93 g/L) and reticulocyte ratio (0.32%) increased slightly and gradually with the improvement of weakness after prednisone (80 mg/day orally, single dosage in the morning) for one week. After prednisone therapy for 2 months, however, Hb decreased (52 g/L) and ‘buffalo hump’ obesity appeared with the reduction in prednisone. Cyclosporin A (25 mg/day, orally, 2 times/d, maintained blood concentration at 200 ng/ml) was added to the prednisone therapy. Cyclosporin A resulted in an increase in Hb to 75-80 g/L after one month,
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although the digestive side-effects, such as nausea and anorexia due to cyclosporin A, became marked. Cyclosporin A was then changed to rapamycin (6 mg orally on the first day and 2 mg/day thereafter). Hb increased to 82 g/L and reticulocytes to 47×10^9/L with significant improvement in the feelings of well-being of the patient after rapamycin for 2 weeks. The other three cases also responded well to rapamycin with Hb increased by 20-40 g/L and without significant side-effects.

**Minimal symptom T-LGLL with indolent course**

We treated three T-LGLL cases (2 males and one female, median age 49 years) character-
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ized by minimal symptoms and indolent disease course for more than three years. The increase in peripheral lymphocyte ratio (50%-80%) and count (2.5-6.1×10^9/L) with moderate splenomegaly and minimal symptoms, such as mild fatigue, were found during routine physical check-ups. The symptoms lasted for one to two years. Routine blood examinations showed normal WBC, Hb and PLT counts. In case 5, laboratory examinations revealed serum rubella DNA (−), CMV DNA (−), EBV DNA (−), HSV DNA (−), antinuclear antibodies (−), ASO (−), rheumatoid factor (−), and CRP (−). Blood chemistry, levels of IgG, IgA, IgM, and complement factors were also normal. In the bone marrow, granulocytes were 59.5% (promyelocytic cells 3.5% with a high proportion of myelocytes) and mature lymphocytes were 33%, with an increased proportion of large granular lymphocytes. Flow cytometric analysis of bone marrow cells from case 5 demonstrated a high proportion of T-cell (48.3%), a low proportion of B-cells and nucleated RBC. Most of the T-cells were CD3+ (99.1%), CD5+ (99.4%), and CD3+CD5+ TCRαβ+ (93%). Flow cytometric analysis of bone marrow cells from cases 6 and 7 showed similar changes. The TCRαβ+ cells were further analyzed using the 24 antibodies for specific detection of the 24 members of the TCRβ family. The ratio of TCRβ14/TCRαβ+ cells was 38.3% in case 5, while the ratio of TCRβ1/TCRαβ+ cells was 36.3% in case 6, and the ratio of TCRβ22/TCRαβ+ cells was 30.14% in case 7 (Figure 2G-I). TCRβ gene rearrangement was positive in one case, and TCRγ gene rearrangement was positive in another two cases. Chronic T-LGLL was then diagnosed in the three cases based on the hematological findings, especially those obtained by flow cytometry. No therapeutic measures were offered to the patients, although routine bone marrow and blood examinations were performed every two months. Disease in these three cases was stable without progression for 2-3 years.

Discussion

T-LGLL is characterized by an abnormal increase in total lymphocyte numbers, particularly the LGLs. The main forms of this disorder are T-LGLL with PRCA and indolent T-LGLL, which are defined according to their clinical characteristics. Here, we report the cases of seven T-LGLL patients with unusual clinical presentations. In four cases, PRCA was the main abnormality, manifested as severe anemia without neutropenia or autoimmune diseases and responding poorly to conventional therapy. In three cases, the symptoms were trivial and insidious without progression for 2-3 years. Ethnic factors can affect the symptoms of T-LGLL significantly. Our findings are similar to those reported by Zhou et al., in which 14 of 34 Chinese T-LGLL patients had PRCA [5]. Kwong reported that among 272 cases T-LGLL in Western countries, the anemia rate was 47% and disease accompanied by PRCA occurred in only 6/143 cases (4%), while the incidence of neutropenia and splenomegaly was as high as 62% and 40%, respectively. Furthermore, in Western countries, T-LGLL accompanied by rheumatoid arthritis and recurrent bacterial infection were significantly higher proportion of, respectively 27% and 30% compared with respectively 4% and 3% in eastern countries [6]. This suggests that the clinical features of T-LGLL differ between eastern and western populations, with eastern populations being more susceptible to the development of PRCA, and western populations are susceptible to neutropenia and autoimmune diseases which differences could be due to racial variations.

In the three patients with minimal symptoms and indolent disease course, we found higher peripheral lymphocyte ratios and numbers of LGLs in blood smears, moderate splenomegaly, high ratios of CD3+CD8+ T-cells, and monoclonal expansion of TCRβ14, TCRβ1 or TCRβ22 cells in bone marrow. These results are consistent with the description of T-LGLL in the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2008. However, a long-term follow-up study indicated that patients having only an indolent disease course that is stable for a long period of time may be psychologically overburdened by the term “leukemia”. Some patients may be inappropriately treated with rigorous chemotherapy for leukemia. The use of the relatively ambiguous term “chronic lymphocyte proliferative disease” to replace the term T-LGLL for this type of T-LGLL remains to be discussed.

Clonal expansion of lymphocytes (>30% monoclonal) is a critical change that is useful for the diagnosis of T-LGLL. Rearrangement of T-cell receptors is also an important indicator of T-cell leukemia, but false positive results limit its use [7]. For the determination of lymphocyte clonal-
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ity, flow cytometry by immunophenotyping and subtyping of TCRβ using the antibodies against the 24 members in TCRβ family is more sensitive and accurate than detection of TCR rearrangements [8-10]. In this study, two of the four patients with PRCA had clonal expansion of TCRβ13.6 cells, although the influence of TCRβ13.6 cells on erythropoiesis is an interesting and unknown issue. In a recent study, 31.4% of T-LGLL patients carried mutations in the STAT3 gene, which may relate to the pathogenesis of T-LGLL [11]; however, the mechanism remains to be elucidated.

Morphological changes in lymphocytes are also an important clue for the diagnosis. T-LGLL should be considered when the increase in large granular lymphocyte ratio and number (>2.0×10⁹/L) in peripheral blood in repeated detection lasts more than 6 months [12]. The large granular lymphocyte number may not be increased (≤2.0×10⁹/L) in 20%-30% of T-LGLL cases. Higher (>2.0×10⁹/L) and lower (≤2.0×10⁹/L) large granular lymphocyte counts may have similar clinical and laboratory findings [13]. When large granular lymphocytes are morphologically atypical, clonal expansion of lymphocytes with a specific immunophenotype should be emphasized to identify the disease. T-LGLL is frequently seen in the elderly, but oligoclonal proliferation of large granular lymphocytes can also be found in normal elderly patients. In elderly patients with large granular T-lymphocytes, symptoms, routine blood examinations, and lymphocytes clonality detected by flow cytometry, these parameters should be re-assayed after 6 months to differentiate T-LGLL from normal changes.

The choice for T-LGLL treatment is immunosuppressive drugs. Cyclosporin A is effective for T-LGLL patients with anemia or PRCA with low cytotoxicity and high safety. The overall response rate is 82.1% and hematological remission rate is 57.1% [14]. The therapeutic protocol of cyclosporin A or cyclosporin A combined with corticosteroids is clinically effective, but relapse occurs frequently during the corticosteroid tapering period [15]. In such cases, re-administration of the previous protocol or a change to other immunosuppressive drugs may still be effective, suggesting that T-LGLL with PRCA requires long-term immunosuppressive treatment. For patients who are unresponsive to routine treatment protocols, we propose that rapamycin can be used to inhibit CD8+ cell and improve clinical symptoms, including low erythropoiesis due to the activated T-cells [16]. Several studies have demonstrated that bortezomib or lenalidomide achieved complete remission for refractory T-LGLL unresponsive to immunosuppressive therapy [17]. Most types of T-LGLL belong to the chronic and indolent neoplasm type, and approximately one-third of the patients are asymptomatic. Some patients achieve long-term survival, with the median survival period exceeding 10 years [18].

In conclusion, in the Asian population, T-LGLL associated with PRCA and minimal symptom T-LGLL were the two most common types of T-LGLL. Detection of clonal expansion of T-lymphocytes through flow cytometric subotyping of the TCRβ family on T-cells is helpful in diagnosing these forms of the disease. Immunosuppressive therapy combined with rapamycin can achieve good effects in patients with T-LGLL associated with PRCA.

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Disclosure of conflict of interest

None.

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