Association of pure red cell aplasia with T large granular lymphocyte leukaemia

Y L Kwong, K F Wong

Abstract

**Aim**—To define the relation between T large granular lymphocyte (T-LGL) leukaemia and pure red cell aplasia in Chinese patients.

**Methods**—Patients with T-LGL leukaemia were identified from a consecutive series of Chinese patients with chronic lymphoproliferative disorders. The diagnosis of T-LGL leukaemia was based on typical morphological and immunophenotypical features, and confirmed by the detection of clonal T cell receptor gene rearrangement. The clinicopathological features, response to treatment, and long term follow up were also examined.

**Results**—Five patients were identified as having T-LGL leukaemia from a consecutive series of 33 Chinese patients with chronic lymphoproliferative disorders. The median follow up time was 45 months. An obvious lymphocytosis was present in only two cases, although an increase in large granular lymphocytes in the peripheral blood was found in four. In one case, the LGL count was within the normal range. Epstein–Barr virus encoded early nuclear RNA was negative in all the cases. There was no evidence of rheumatoid arthritis, and none of the patients presented with recurrent infections. On follow up, pure red cell aplasia occurred at some stage of the disease in all the patients. This responded to treatment with cyclosporin A in two and with antithymocyte globulin in one. Two patients remained transfusion dependent.

**Conclusions**—In contrast to Western patients, Chinese patients with T-LGL leukaemia do not appear to suffer from rheumatoid arthritis and recurrent infections, but pure red cell aplasia is a major cause of morbidity in this ethnic group.

**Keywords:** pure red cell aplasia; large granular lymphocyte leukaemia

T large granular lymphocyte (T-LGL) leukaemia is a rare chronic lymphoproliferative disorder of T cells characterised by anaemia, neutropenia, and a persistent increase in large granular lymphocytes, which are large lymphocytes with a low nucleus to cytoplasm ratio and abundant cytoplasm with azurophilic granules. T-LGL leukaemia is an uncommon hematological disorder in the West, accounting for 2–5% of all chronic lymphoid leukaemias. Common clinical associations include rheumatoid arthritis and granulocytopenia; autoimmune cytopenias also occur, but less commonly, while pure red cell aplasia is a rare association.

On the other hand, T-LGL leukaemia is apparently more common in the East. In one Chinese series it accounted for 9% of all chronic lymphoproliferative disorders. It is also thought to be relatively common in the Japanese, although the exact incidence remains undefined. Oriental patients with this condition tend to present with different features from occidental patients—for example, rheumatoid arthritis is very rare but pure red cell aplasia appears to be much more common.

We present the clinicopathological features of five consecutive Chinese patients with T-LGL leukaemia and report a high frequency of pure red cell aplasia in this population.

**Methods**

**Patients**

All the patients were ethnic Chinese. The diagnosis of T-LGL leukaemia was based on the presence of large granular lymphocytes in the peripheral blood and confirmed by immunophenotyping. Clonal rearrangement of the T cell receptor γ (TCRγ) gene must also be demonstrable. The diagnosis of pure red cell aplasia was based on anaemia, reticulocytopenia (<0.1%), and selective erythroid hypoplasia/aplasia in a normocellular marrow with normal myeloid and megakaryocytic cell lines. Vitamin B-12 and folate deficiencies were excluded. A remission of the pure red cell aplasia was defined as an untransfused haemoglobin of > 10 g/dl and the return of a normal reticulocyte count. The clinical features of cases 1 to 3 had been reported briefly elsewhere.

**Immunophenotyping, TCRγ gene rearrangement, and in situ hybridisation**

Immunophenotyping was performed on peripheral blood or bone marrow mononuclear cells by the biotin–streptavidin method. The mononuclear cells in the peripheral blood or bone marrow were concentrated by Ficoll density gradient sedimentation. Cytospin slides were prepared from the mononuclear cell suspension. Membrane staining was interpreted together with cellular morphology, while one of the cytospin slides was simultaneously Giemsa stained and examined. The polymerase chain reaction (PCR) was used to demonstrate a clonally rearranged TCRγ gene, according to McCarthy et al. Two pairs of Vγ primers (Vγ: 5′-TCT GG(G/A) GTC TAT TAC TGT GC-3′; Vγ: 5′-CTC ACA CTC (C/T)CA CTT C-3′), complementary to consensus sequences...
of V-I and V-II/IV families of the TCRγ gene, respectively) and two pairs of Jγ primers (Jγ: 5'-CAAGTGTTGTTCCACTGCC-3'; Jγ: 5'-GTTCCTATGAGC(T/C)TAGTCC-3', 95 base pairs for the V1/V2 J1 primer pairs, and 80 to 110 base pairs for the Vγ/Vγ J1 primer pairs. In situ hybridisation for Epstein–Barr virus encoded early RNA (EBER) was performed on paraffin embedded marrow biopsies or peripheral blood mononuclear cell cytospin preparations as previously described.10

Results

CLINICOPATHOLOGICAL FEATURES

Four male patients and one female were diagnosed as having T-LGL leukaemia among 33 consecutive unselected patients with chronic lymphoproliferative diseases who presented in a six year period (1991 to 1996). The clinicopathological features of these patients are shown in table 1. The median age at presentation was 51 years (range 47 to 73).

The initial symptoms were anaemia in four cases and peripheral neuropathy in one. Recurrent infection was not observed in any patient. Neither clinical nor serological evidence of rheumatoid arthritis was present in any of the cases. At presentation, an increase in the percentage of large granular lymphocytes was found in four cases, the absolute LGL count ranging from 70 to 110×109/l. These cells showed considerable variation in size and morphology (fig 1). Bone marrow examination of these patients showed a variable infiltration by large granular lymphocytes, ranging from 25% to 45%. The direct antiglobulin test was negative. Pure red cell aplasia was present in two cases at presentation. Case 3 did not have an increase in LGL count in the peripheral blood and marrow at any stage of her disease, and this accounted for the original diagnosis of idiopathic pure red cell aplasia. T-LGL leukaemia was suspected three years after presentation because of the presence of LGL cells of atypical morphology (fig 1). In the other three cases, pure red cell aplasia developed six, 10, and 26 months after presentation.

IMMUNOPHENOTYPING, TCRγ GENE REARRANGEMENT, AND IN SITU HYBRIDISATION

Immunophenotypic studies showed that the large granular lymphocytes expressed the immunophenotype CD2+CD3+CD4−CD8+, CD2+CD3+CD56− in all cases. PCR for the TCRγ gene showed clonal rearrangements in all (fig 2). In cases 3 and 4, two bands were amplified consistently with the biallelic TCRγ gene rearrangements that occur in about 40% of T cell malignancies. In case 3, PCR was performed retrospectively on the marrow obtained initially at the diagnosis of pure red cell aplasia, and showed that a clonal population of T cells

Table 1  Clinicopathological features on presentation and treatment outcome in five Chinese patients with T large granular lymphocyte (LGL) leukaemia

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/age</th>
<th>Hb (g/dl)</th>
<th>WBC (×109/l)</th>
<th>Platelet (×109/l)</th>
<th>Marrow LGL (%)</th>
<th>Immunophenotype of LGL</th>
<th>Associated disease (months from diagnosis)</th>
<th>Response of PRCA to treatment (months or courses given)</th>
<th>Follow up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/47</td>
<td>6.5</td>
<td>15.0 (65)</td>
<td>145</td>
<td>37</td>
<td>CD2+CD3+−ve CD4−CD8+CD56− PRCA (6)</td>
<td>NR (4) NR (1) NR (6) PRCA (6)</td>
<td>Myleran† NR (6) Myleran‡ Transfusion independent (45)</td>
<td>Transfusion independent (55)</td>
</tr>
<tr>
<td>2</td>
<td>M/48</td>
<td>6.0</td>
<td>20.0 (58)</td>
<td>360</td>
<td>45</td>
<td>CD2+CD3+−ve CD4−CD8+CD56− PRCA (26)</td>
<td>NR (4) NR (1) NR (6) Myleran‡ NR (6) Myleran‡ Transfusion dependent (55)</td>
<td>Transfusion dependent (55)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F/73</td>
<td>5.8</td>
<td>4.0 (2)</td>
<td>150</td>
<td>Not incr</td>
<td>CD3+CD4−−ve CD8+ PRCA (P)</td>
<td>NR (6) NR (1) NR (3) PRCA (10) peripheral neuropathy (P)</td>
<td>– – R (9) – –</td>
<td>Transfusion independent (49)</td>
</tr>
<tr>
<td>4</td>
<td>M/51</td>
<td>4.7</td>
<td>2.5 (27)</td>
<td>161</td>
<td>30</td>
<td>CD2+CD3+−ve CD4−CD8+CD56− PRCA (P)</td>
<td>– – R (9) – –</td>
<td>– – Transfusion independent (12)</td>
<td>Transfusion independent (24)</td>
</tr>
<tr>
<td>5</td>
<td>M/50</td>
<td>13.9</td>
<td>4.1 (54)</td>
<td>225</td>
<td>25</td>
<td>CD2+CD3+−ve CD4−CD8+CD56− PRCA (10)</td>
<td>– – R (3) – –</td>
<td>– – Transfusion independent (24)</td>
<td>– –</td>
</tr>
</tbody>
</table>

ATG, antithymocyte globulin (Atgam, Upjohn; 25 mg/kg/d × 5); CsA, cyclosporin (8 mg/kg/d); CTX, cyclophosphamide (100 mg/d); DCF, deoxycoformycin (4 mg/kg/course); EBER, Epstein–Barr virus encoded early RNA; Hb, haemoglobin (g/dl); HDM, high dose methylprednisolone (1 g/d × 3); IVIg, intravenous immunoglobulin (40 mg/kg/d × 5); incr, increased; NR, non-remission, transfusion dependent; P, on presentation; PRCA, pure red cell aplasia; R, remission (Hb > 10 g/dl, transfusion independent); WBC, white blood cell count (×109/l).
†Prednisone 1 mg/dl.
‡Myleran dose 4–6 mg/d.

Figure 1  Morphological variation of cells in T large granular lymphocyte (T-LGL) leukaemia. (A) Typical LGL with voluminous cytoplasm containing coarse granules; (B) LGL with pale vacuolated cytoplasm containing very fine granules; (C) LGL showing numerous cytoplasmic villi; (D) small LGL with a central nuclear cleft, easily mistaken as an ordinary small lymphocyte unless examined under high power. (Wright stain, ×867.)
already existed despite the absence of an increase in large granular lymphocytes. In situ hybridisation for EBV EBER was negative in all the cases.

TREATMENT OUTCOME

In three patients who presented with pure red cell aplasia, the initial treatment was corticosteroids followed by a variety of other agents including high dose steroids, cyclophosphamide, cyclosporin A, intravenous immunoglobulin, and antithymocyte globulin (table 1). Two were refractory to treatment, but one responded to antithymocyte globulin and became transfusion independent. In two other patients, the initial treatment was cyclosporin A, starting at 8 mg/kg/day in two divided doses. Both achieved a remission. The LGL count in cases 1 and 2 decreased to less than $2 \times 10^3/\mu l$, with treatment, while in cases 3–5 it remained stable.

Discussion

This series illustrates several clinicopathological differences between oriental patients with T-LGL leukaemia and their occidental counterparts. First, the overall frequency of T-LGL leukaemia was 15% in this series from a single tertiary referral centre, which is higher than that reported in our previous multicentre study of Chinese patients with chronic lymphoproliferative disorders (9%). This might be attributable to a difference in referral patterns. However, both frequencies are higher than the figure of 2–5% in Western series. Second, none of our cases had clinical or serological evidence of rheumatoid arthritis. In fact, rheumatoid arthritis was not observed in any of the patients with T-LGL leukaemia reported in two Japanese and Chinese series. Again, this is in contrast to Western series, where there is serological evidence of rheumatoid arthritis in as many as 30–50% of cases. Third, recurrent infections were not the presenting symptoms and were not a major problem in any of our cases, nor in any of the previously reported oriental patients.

On the other hand, pure red cell aplasia was observed in all our patients. These findings are in agreement with data on Japanese patients with T-LGL leukaemia, in whom the frequency of pure red cell aplasia is as high as 64%. During our study period, another four cases of pure red cell aplasia were seen (one was idiopathic, the others secondary to myeloma, B chronic lymphocytic leukaemia, and myelodysplasia; unpublished observations). Therefore, nearly half of all our patients with pure red cell aplasia had T-LGL leukaemia. These findings indicate a close association between T-LGL leukaemia and pure red cell aplasia in oriental patients. This association is thought to be very uncommon in occidental ethnic groups. However, a recent report on cases of pure red cell aplasia studied for TCR gene rearrangement in occidental patients showed that 19% had T-LGL leukaemia, suggesting a more common association than was previously recognised. Furthermore, as only some of the patients had been investigated by molecular methods in that study, the actual frequency might be higher. The importance of this observation is that in oriental patients, and perhaps also in occidental patients, an underlying T-LGL leukaemia should be excluded in the diagnostic evaluation of pure red cell aplasia.

Two of our cases had only modest increases in LGL counts, making the diagnosis of T-LGL leukaemia difficult. This is illustrated in case 3 who had no increase in LGL count, although a clonal T cell proliferation was already demonstrable by PCR in the initial diagnostic marrow sample. In these cases, abnormal morphology of the large granular lymphocytes and nuclear atypia might be a clue, but the definitive diagnosis depended on the demonstration of a clonal expansion of T cells. It is therefore important to recognise that lymphocytosis may not be obvious in patients with T-LGL leukaemia, and unless the blood film is examined carefully and periodically the diagnosis may be overlooked, as about 8% of cases of T-LGL leukaemia have an LGL count of less than $1 \times 10^3/\mu l$. Furthermore, molecular studies for clonal T cell proliferation should be performed in clinical situations where T-LGL leukaemia is a diagnostic possibility.

No evidence of EBV infection was demonstrable in any of our cases, consistent with the observations from other reports. This suggests that EBV is not involved in the pathogenesis of T-LGL leukaemia, even in a population like ours where EBV is known to be associated with many malignancies such as nasopharyngeal carcinoma and nasopharyngeal lymphoma.

Finally, although a remission of the pure red cell aplasia was achieved in three cases after treatment, the T-LGL leukaemia remained unchanged. In two other patients, treatment of the red cell aplasia was unsatisfactory, despite the use of cyclophosphamide and cyclosporin—reported to be the most effective treatment. New treatments are needed to avoid the long term complications of transfusion. We are currently evaluating the use of purine analogues including deoxycoformycin in some of our patients.

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