CD3 + CD8+ T cell lymphocytosis masking B cell leukaemia

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Summary  A patient with CD3, CD8 positive lymphocytosis presented with features consistent with T cell chronic lymphocytic leukaemia/proliferations of large granular lymphocytes. The marrow and blood lymphoid populations (19.4 × 10⁹/l) contained more than 80% CD3 and CD8 positive cells with no evidence of a monotypic B cell population. A biopsy specimen of a vasculitic rash showed a diffuse infiltrate of CD3, CD8 positive cells into the upper dermis, consistent with T cell lymphocytic disease. After follow up for two years without treatment the blood lymphocyte count was 53 × 10⁹/l and was composed of cytologically small lymphocytes. A monoclonal SIg M D k lymphoid population (more than 90%) was demonstrable in sample blood and marrow aspirate. Gene rearrangement studies carried out on DNA extracted from peripheral blood lymphocytes at presentation and at two year follow up exhibited JH and Ck immunoglobulin gene rearrangement but no rearrangement of T cell receptor TcRγ and β genes.

It is thought that this is the first well documented case of an aggressive CD8 positive lymphocytosis preceding, or in response to, an underlying B cell neoplasm.

T cell lymphocytosis may occur as part of a well characterised T cell malignancy such as T prolymphocytic leukaemia, T acute lymphoblastic leukaemia, adult T cell leukaemia lymphoma, or Sézary syndrome. A further group of patients with chronic T cell lymphocytosis (CTCL), have a moderate increase in large granular lymphocytes with a CD3, CD8, Leu7, FCγ positive and CD5 negative phenotype. These patients often have concomitant rheumatoid arthritis, splenomegaly, and neutropenia which have an indolent clinical course. Most cases, but not all, show rearrangement of the T cell receptor (TcRβ) gene.

We report an unusual case of CTCL in which the proliferating cells were initially CD2, CD3, CD8 positive but were morphologically pleomorphic and showed no TcRβ rearrangement. Subsequently the T cells were replaced in blood and bone marrow by small clefled cells that were morphologically and phenotypically B cells.

Material and methods

Analyses were performed on samples of peripheral blood, bone marrow, cerebrospinal fluid and a skin biopsy. Mononuclear cells were isolated from blood and bone marrow over Ficoll-Hypaque, preincubated for one hour at 37°C to remove absorbed immune complexes and then analysed immediately for various membrane determinants. SIg was shown by direct immunofluorescence using rabbit anti-Ig heavy and light chain reagents (Dako Immunoglobulins, Copenhagen, Denmark) by standard methods; reagent specificity has been described previously. SIg light chain determinants were also shown by an indirect immunoperoxidase method using monoclonal anti-Igκ or anti-Igλ followed by peroxidase conjugated rabbit anti-mouse Igs (Dako Immunoglobulins) using methods described previously.

The following monoclonal antibodies were used with the “cluster of differentiation” or CD nomenclature as allocated by the first, second, and third monoclonal antibody workshops (shown in parentheses): OKT11 (CD2), UCHT1 (CD3), OKT3 (CD3), OKT4 (CD4), HB2 (CD7), OKT8 (CD8), B1 (CD20), WR17 (CD37), RFB4 (CD22) and WR18. Both UCHT1 and OKT3 detect T3 molecules which are present on all mature T cells. OKT1 recognises most normal T cells and a minor normal B subset.
Anti-B1 binds to an antigen limited to cells of the B cell compartment. The full specificity (CD22) of RFB4 has been described by Campana et al. WR18 is a marker for HLA class IIβ chain determinant (K Moore, Regional Immunology Unit, Southampton General Hospital, personal communication).

Binding of monoclonal antibodies to isolated mononuclear cells was shown by indirect immunofluorescence using fluorescein-conjugated, affinity purified F(ab')2, sheep anti-mouse IgG (Sigma) and by indirect immunoperoxidase staining using peroxidase conjugated polyvalent rabbit anti-mouse Ig (Dako Immunoglobulins), followed by Romanowsky staining to identify cells by morphological features. All first layer monoclonal antibodies were microfuged for 15 minutes immediately before use. Lymphocytes sensitised with an irrelevant mouse monoclonal antibody were always completely negative when stained with the fluorescent or peroxidase conjugated second antibody.

Methods for the immunostaining of formalin fixed, wax embedded and frozen sections have been described previously. Sheep red cell rosette forming cells were assayed as previously described. Analysis of frozen sections of a skin biopsy

**Fig 1** Peripheral blood lymphocytes: (a) large granular lymphocyte; (b) cells with clefsed nuclei; (c) and (d) large cells with basophilic cytoplasm and nuclei.
specimen were completed with a panel of monoclonal antibodies (CD3, CD4, CD8, CD37, IB5 (HLA class II), KiM4B (follicular dendritic cells) and E11 (C3b receptor)) using the immunoperoxidase technique described previously.7

**GENE REARRANGEMENT STUDIES**

DNA was extracted from peripheral blood lymphocytes by routine methods and digested with restriction endonuclease.13 The DNA fragments were subjected to electrophoresis in a 0.7% agarose gel and transferred to nylon filters (Hybond-N) by Southern blotting. Filters were hybridised with 32P probes specific for immunoglobulin and T cell receptor genes, washed under appropriate conditions, and subjected to autoradiography. The three probes used in the study were an immunoglobulin heavy chain joining region JH probe (C76R51A) and immunoglobulin \(\kappa\) chain probe C\(\kappa\) (pUCR17Ck)15 and a TcR\(\beta\) gene probe (Jurkat 1 2) and a TcR\(\gamma\) gene probe (M13H60).1617 DNA samples were hybridised with the JH probe after digestion with BamHI Hind III and EcoRI and the C\(\kappa\) probe after digestion with BamHI. Hybridisation with the TcR\(\beta\) and \(\gamma\) chain probes was carried out after DNA digestion with EcoRI, Hind III, and Bam HI.18

**ASSAY OF Ig EXPORT**

For culture, lymphocytes were suspended at 2 \(\times\) 10⁷ ml⁻¹ containing non-essential amino acids, L-glutamine, penicillin and streptomycin and 10% fetal calf serum, swirled gently at 37°C, and samples taken at intervals for assessment of Ig production over a

**Table**  
**Cell marker and gene rearrangement data**

<table>
<thead>
<tr>
<th>Date</th>
<th>Tissue</th>
<th>Lymphocytes (10⁹/l)</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD7</th>
<th>CD8</th>
<th>HLA class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>*5/83</td>
<td>Peripheral blood</td>
<td>19-4</td>
<td>70</td>
<td>50</td>
<td>70</td>
<td>10</td>
<td>---</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
<td>↑</td>
<td>74</td>
<td>---</td>
<td>62</td>
<td>2</td>
<td>---</td>
<td>80</td>
</tr>
<tr>
<td>3/85</td>
<td>Peripheral blood</td>
<td>59</td>
<td>---</td>
<td>23</td>
<td>10</td>
<td>1</td>
<td>---</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bone marrow</td>
<td>↑</td>
<td>---</td>
<td>2</td>
<td>1</td>
<td>---</td>
<td>---</td>
<td>95</td>
</tr>
<tr>
<td>*3/86</td>
<td>Peripheral blood</td>
<td>28-7</td>
<td>---</td>
<td>---</td>
<td>90</td>
<td>---</td>
<td>48</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Cerebrospinal fluid</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>85</td>
</tr>
</tbody>
</table>

*Peripheral blood cells on 11/83 and 4/86 were probed for TcR\(\gamma\); on both occasions germline bands were observed with Bam H1 (15-0 and 12-0 kb).
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period of five and a half hours. The Ig exported was assessed by enzyme linked immunosorbent assay (ELISA).11

ASSAY OF NATURAL ACTIVITY
Whole blood mononuclear cells were separated out by centrifugation over Ficoll-Hypaque and then washed in Hank's balanced salt solution (calcium and magnesium free). Mononuclear cells were assayed for natural killer activity against the natural killer sensitive cell line, K562, as described by White et al.19 Briefly, 100 ml of effector cells were mixed with an equal volume of 51Cr labelled target cells (10⁵ per ml) at ratios of 80:1, 40:1, and 20:1. All assays were performed in triplicate and incubation proceeded for four hours at 37°C, prior to harvesting and measuring radioactive chromium release. For each set of triplicate cultures the specific lysis was calculated relative to the values for 51Cr release in detergent alone.

CASE REPORT
A 62 year old man first presented in March 1983 with a flu-like illness associated with an apparently vasculitic rash on his legs. He had no lymphadenopathy nor hepatosplenomegaly. His haemoglobin concentration was 14.6 g/dl, white cell count 12.6 × 10⁹/l with 74% lymphocytes, and a platelet count of 54 × 10⁹/l. Lymphocyte morphology was pleomorphic and included 48% small lymphocytes, 24% smear cells, 12% large cells with basophilic cytoplasm and either one or more nucleoli, 10% large granular lymphocytes and 6% clefted cells (fig 1).

Ninety per cent of peripheral blood lymphocytes showed multiple granular positivity with acid phosphatase staining. A bone marrow aspirate was heavily infiltrated with lymphocytes which also showed a T cell phenotype. Chromosomal analysis of peripheral blood and bone marrow yielded normal results. Serum electrophoresis showed a polyclonal mix in gammaglobulin, and circulating immune complexes were detected. No treatment was given at this stage. His subsequent clinical course is summarised in fig 2.

Over the following year he remained well and his peripheral blood white cell count rose to 25 × 10⁹/l. Chromosomal analysis of the peripheral blood was repeated on two occasions and all metaphases obtained using tetradecanoyl phorbol acetate (TPA) as a mitogen showed 46XY del 9q. (q21;q32). All metaphases studied after stimulation with phytohaemagglutinin were normal. His rash waxed and waned and a skin biopsy specimen taken in February 1985 showed an extensive lymphocytic infiltrate affecting both the dermis and epidermis.

In March 1985 he developed a warm-type autoimmune haemolytic anaemia. His blood count showed a haemoglobin of 8.9 g/dl, a white cell count of 53 × 10⁹/l with 90% lymphocytes, and a platelet count of 239 × 10⁹/l. Most of the lymphocytes were small with clefted nuclei and little cytoplasm. A bone marrow aspiration performed at this time showed a diffuse infiltrate of clefted cells. Chromosomal analysis of the peripheral blood was normal with both TPA and PHA.

He was treated with prednisolone and oral cyclophosphamide with a rapid improvement in his haemoglobin, but when the steroids were gradually decreased over the next two months, his haemoglobin again fell to 8.0 g/dl so that he was restarted on prednisolone together with weekly injections of intravenous cyclophosphamide. His haemoglobin concentration again improved and had not deteriorated at the time of writing. He remained well until December 1985, when he developed clinical features of a right hemiparesis and a computed tomography scan showed a right cerebellar lesion. The cerebrospinal fluid contained small numbers of lymphocytes and an IgGk paraprotein. Considerable neurological improvement was obtained with cranial irradiation and oral dexamethasone.

Results

CELL MARKERS
Representative cell marker results on blood, marrow, and cerebrospinal fluid are given in the table. Immunohistological analysis of the skin biopsy specimen showed an infiltrate of CD3 and CD8

<table>
<thead>
<tr>
<th>CD20</th>
<th>CD37</th>
<th>B cell</th>
<th>Gene rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TcRβ</td>
</tr>
<tr>
<td>30</td>
<td>—</td>
<td>—</td>
<td>G</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>3, 23</td>
<td>—</td>
</tr>
<tr>
<td>83</td>
<td>—</td>
<td>78, 10</td>
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</tr>
<tr>
<td>—</td>
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<td>10, 72</td>
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<tr>
<td>—</td>
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</tr>
<tr>
<td>—</td>
<td>—</td>
<td>90</td>
<td>—</td>
</tr>
</tbody>
</table>

|                 | C2   | C1   |
|                 | —    | —    |
positive cells (fig 3). The infiltrate was negative for the pan B reagent WR17 and HLA class II. Staining with E11 (C3b receptor) was negative.

GENE REARRANGEMENT
DNA isolated from the initial and second peripheral blood samples (05/83 and 03/85, respectively) showed rearranged Jh genes and progressive loss of the germline configuration (fig 4). Using the CK probe, DNA from both blood samples digested with BamH1 showed biallelic deletion of the 12-0 kb CK germline band and two rearranged bands. DNA from all samples were digested with BamH1, Hind III, and EcoRI restriction endonucleases then hybridised to the labelled TcRβ probe. One germline Bam H1 (24 kb) three germline Hind III (8-0, 6-5, and 3-5 kb), and two germline EcoRI bands (11-5 and 4-0 kb) were identified in all samples. Germline bands were also observed with TcRγ (table).

IG STUDIES
Cells in culture at the time that SIgκ positive cells predominated produced κ light chains (13 ng/10² cells/4 hour culture). Assays for λ light chain, μ, and γ heavy chains did not suggest high production over a four hour culture period. Cerebrospinal fluid analysed by isoelectric focusing contained a trace of IgGκκ paraprotein. No paraproteins could be shown in serum or in concentrated urine by this technique.

NK CELL ASSAYS
When tested on two occasions in May 1983, mononuclear cells failed to produce specific lysis of the natural killer sensitive target cell line K562 although, on both occasions, good levels of specific lysis were obtained with whole mononuclear fractions from normal volunteers.

Discussion
The evolution seen in this case is remarkable. On presentation a predominant population of lymphocytes of T cell phenotype were present in blood and bone marrow. In blood a minor population of CD20 positive SIg negative B cells were detectable, comprising 30% of the total lymphoid population. While CD20 staining was not completed in the marrow, the residual B cell population was polyclonal. The initial picture immunophenotypically resembled that seen in CTCL and this was supported by a subsequent skin biopsy which showed an infiltrate of CD3 positive, CD8 positive, and CD37 negative cells. In contrast to most cases of CTCL, the neoplastic cells were Igκκ positive, however, there were no TcRβ or γ gene rearrangements detectable.

The possibility that this neoplasm was a CD3 positive B cell tumour was excluded by the failure of the infiltrating CD3 positive and CD8 positive cells in the skin and blood to mark for CD37 and CD20, respectively. Furthermore, in the CD3 positive B cases reported by us, all the neoplastic B cells were Igκκ positive. The emergence of a clinically silent B cell neoplasm was confirmed by the follow up. These rearrangements were accompanied by a progressive loss of the germline configuration. At presentation one rearranged band was evident with the Jκ probe; this band was also seen at follow up together with the
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![Fig 4](image-url)

**Fig 4** Autoradiograph of DNA digested with Bam HI and Hind III. hybridised with the J, probe.

Track C: normal DNA (germline bands at 7.5 kb (Hind III) and 17.0 kb (Bam H1)).

Tracks 2 and 3: DNA obtained from peripheral blood lymphocytes at 5/83 and 3/85, respectively (Note progressive loss of germline bands for both enzymes and appearance of rearranged bands at 6 kb and 26 kb (Hind III) and at 14 kb (Bam H1). In the Hind III digest the 26 kb rearranged band was not present at 5/83 while the 6 kb rearranged band was present in both 5/83 and 3/85 samples.

The clinical importance of the chromosomal abnormality del 9q (q 21; q 32) detected with TPA stimulation on two occasions during the period of chronic T cell lymphocytosis is unclear. Chromosome abnormalities have not been reported regularly in CTCL and this particular deletion is not characteristically associated with either B or T cell malignancy.

Two possible explanations for this unusual pattern of B cell tumour evolution may be advanced. First, the original T cell proliferation may simply have been a response to an unspecified viral infection in a patient with an occult B cell neoplasm. The prolonged persistence of the T cells and the T cell infiltrate in the skin make this unlikely. Alternatively, the T lymphocytosis may have represented a vigorous host response to a B cell neoplasm. The initial flu-like illness and vasculitic rash and accompanying polyclonal gammopathy and circulating immune complexes would have represented the bystander effects produced by release of lymphokines. If this were so, the initial immunoglobulin gene rearrangement would be accounted for by an occult population of neoplastic B cells which were surface Ig negative. The original blood picture showed 6% clefled cells.

Increased numbers of CD8 positive cells are a well recognised feature of B-CLL and in a case of B-CLL reported by Liberati et al T suppressor lymphocytosis appeared 11 months before the eventual appearance in the blood of monotypic B cells, although in this case no gene rearrangement studies were performed. Early B cell neoplasms are usually clinically silent so that it is impossible to know how frequently a T cell lymphocytosis is seen before the appearance of neoplastic B cells. None of the reported cases of CTCL in which TcR genes were not rearranged have shown an evolution reported in this case.

**References**

I. Phenotypic differences of B lymphocytes in the bone marrow and peripheral lymphoid tissue. *J Immunol* 1985;134:1524.


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