Studies on blood lymphocytes of patients with nodular poorly differentiated lymphocytic lymphoma

AS KRAJEWSKI, AE DEWAR

From the Department of Pathology, University Medical School, Teviot Place, Edinburgh

SUMMARY  T and B lymphocytes were measured in pretreatment blood samples from patients with nodular poorly differentiated lymphocytic lymphoma (NPDLL). There were significant differences in T cell values between control groups and patients with NPDLL. In 13 out of 20 cases of NPDLL, blood lymphocytes showed abnormalities of immunoglobulin light chain expression and were considered to show an abnormal clonal expansion of B lymphocytes. The abnormal clone of B cells in the blood reflected that found in lymph nodes and could be detected in the absence of bone marrow involvement or blood lymphocytosis.

The B cell origin of the majority of non-Hodgkin's lymphomas (NHL) has been established using surface markers. In most cases the investigations into the circulating cells in these patients have been incidental to the studies on the tumour cells from solid tissues. Garrett et al. reported that the blood lymphocytes of many patients with NHL had detectable abnormalities of surface immunoglobulin light or heavy chain expression; but the numbers of each sub-type of NHL in this series were small. Ligler et al. found that seven out of eight cases of NHL had detectable abnormalities in surface immunoglobulin in the circulating B cell population.

This paper describes the results of investigations into the peripheral blood lymphocyte subpopulations in cases of nodular poorly differentiated lymphocytic lymphoma (NPDLL). The findings are correlated with clinical stage and, in some cases, with laboratory studies on tumour tissue.

Material and methods

Solid tumour samples, mainly lymph nodes, were obtained as fresh specimens from surgical theatres in hospitals in the Lothian Region. Blood samples were taken either just before biopsy or 2 weeks after biopsy. All blood samples were taken pretreatment. The control material in this study consisted of samples of lymph node tissue and blood from patients who were found not to have lymphoma—for example, reactive hyperplasia, toxoplasmosis, carcinoma etc. Patients histologically diagnosed as nodular lymphoma were staged according to Ann Arbor classification and grouped as either stage I-III or stage IV. Cell suspensions were obtained from lymph nodes and peripheral blood as previously described.

Lymph nodes were teased into TC 199 (Flow Laboratories, Irvine, Ayrshire) and washed once before surface marker studies. Blood mononuclear cells were isolated using Ficoll/Hypaque and washed twice before use. In surface marker studies we used sheep erythrocyte rosettes to identify the T cell population. B cells were counted by means of an indirect fluorescence method on mononuclear cells which had been incubated in serum-free medium for 45 min at 37°C and then washed for a third time. Unconjugated rabbit antiserum to human sera, kappa and lambda chains were obtained from Nordic Immunologic Laboratories. The sera were adsorbed for 30 min at 37°C with washed human erythrocytes before ultracentrifugation at 150 000 g for 40 min, aliquoting, and storage at −70°C. Goat anti-rabbit IgG conjugated with FITC, from the same source, was also ultracentrifuged before storage at −70°C. 3-5 × 10⁶ cells were incubated for 10 min at 37°C in the appropriate dilution of specific antiserum, washed once in 10 ml phosphate buffered saline (PBS) then incubated in the FITC conjugate for 20 min on ice, washed once in 10 ml PBS and examined under ultraviolet illumination on a Leitz Orthomat with incident illumination.

We used immunoperoxidase techniques to demonstrate the monoclonality of the tumour in paraffin sections of the lymph nodes, as described by Burns. Sections were stained for IgM heavy chain and kappa...
and lambda light chains. Blood films, stained with May-Grünwald-Giemsa, were used for routine differential counts and examined for the presence of morphologically abnormal circulating cells.

Results

**T CELLS IN BLOOD**

The results in Tables 1 and 2 show that of 38 cases of NPDLL, 13 had low lymphocyte counts (<1 x 10⁹/l (<1000/mm³)) at time of presentation, six had lymphocytosis (between 5-10 x 10⁹/l (5-10 000/mm³)) whilst three were frankly leukaemic (more than 10 x 10⁹/l (>10 000/mm³)). All groups of patients had significantly lower percentages of T cells than normal donors or patients with non-lymphomatous lymphadenopathy (Student's t test p < 0·0005). There were no significant differences in percentages of T lymphocytes between patients with low and normal lymphocyte counts in either the stage I-III or the stage IV groups.

There was a significant difference (p < 0·0005) between the group stage I-III and stage IV (Fig. 1). Only one of the patients in stage I-III group (Table 1, Case 2) had less than 35 % T cells, whilst 10/14 with stage IV (but without lymphocytosis) had less than 35 % T cells. All the patients with either lymphocytosis or leukaemia had bone marrow involvement and a reduced percentage of T cells.

The results of absolute T cell numbers are summarised in Fig. 2. They were essentially similar to those shown in Fig. 1, except that stage I-III patients with low lymphocyte counts were T cell depleted, and patients with lymphocytosis had, in absolute terms,
normal individuals has a constant pattern; with a kappa:lambda (\(\kappa:\lambda\)) ratio of about 3:2. In 28 blood samples from patients with non-lymphomatous lymphadenopathy (12 samples) and normal donors (16 samples) the mean \(\kappa:\lambda\) ratio was 1.48 with an upper limit (mean + 2 SD) of 2.75 and the mean lambda:kappa (\(\lambda:\kappa\)) ratio was 0.78 with an upper limit of 1.35 (mean + 2 SD). Ratios above these limits were considered to indicate a monoclonal expansion of lymphocytes with either kappa (\(\kappa:\lambda > 2.75\)) or lambda (\(\lambda:\kappa > 1.35\)) light chain. Only one of the 28 non-lymphoma cases (donor attending blood transfusion centre, no repeat sample available) had an abnormal ratio according to these criteria, whilst 9/20 NPDLL patients had an abnormal \(\kappa:\lambda\) ratio and 5/20 had an abnormal \(\lambda:\kappa\) ratio. Abnormal blood light chain ratios were found in 5/6 cases without bone marrow involvement in 9/13 cases with bone marrow involvement. In 11 cases abnormal \(\kappa:\lambda\) or \(\lambda:\kappa\) ratios were found in the absence of morphologically abnormal lymphocytes in routinely-stained peripheral blood films. In three cases with abnormal light chain ratios morphologically abnormal cells were seen in blood films.

Light chain expression on lymph nodes were studied by means of indirect fluorescence on cell suspension. In lymph nodes from 27 patients with non-lymphomatous conditions, the mean \(\kappa:\lambda\) ratio was 1.54 with an upper limit (mean + 2 SD) of 3.70 and the mean \(\lambda:\kappa\) ratio was 0.86 with an upper limit (mean + 2 SD) of 1.70. Only one of the 27 cases had an abnormal ratio according to these criteria. This lymph node showing the features of dermotopathic lymphadenopathy with no evidence of lymphoma. Immunofluorescent studies were carried out on lymph nodes in 11 NPDLL patients; five cases showed an abnormal \(\kappa:\lambda\) ratio and one case an abnormal \(\lambda:\kappa\) ratio, the remaining five cases showed normal proportion of kappa- and lambda-bearing lymphocytes. In the cases with monoclonal expansion the abnormality was consistent with that found in blood. In none of our cases did we detect one light chain in the blood and the other in the lymph nodes (Table 3).

Immunoperoxidase-stained sections were examined in 19/20 NPDLL cases. In eight cases there was definite evidence of monoclonal cytoplasmic light chain expression. Other cases showed either a polyclonal staining pattern or were negative. In cases with a monoclonal staining pattern the abnormality was consistent with immunofluorescence findings in blood and lymph node cell suspensions (Table 3).

In five cases of NPDLL, blood samples were obtained both before and after treatment. Results of receptor studies on blood are shown in Table 4. In three cases (12, 27 and 35) abnormal \(\kappa:\lambda\) or \(\lambda:\kappa\) ratios

### B Cell Population in NPDLL

Light chain expression on the circulating B cells in

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**Fig. 1** Percentage T cells in the blood of NPDLL patients. \(n\) = number of cases in each group, \(\bullet\) = mean, \(\circ\) = individual value. Stage IV cases had normal lymphocyte counts; Stage IV-L cases had lymphocytosis.

**Fig. 2** Percentage T cells in the blood of NPDLL patients. \(n\) = number of cases in each group, \(\bullet\) = mean, \(\circ\) = individual value. Stage IV cases had normal lymphocyte counts; stage IV-L cases had lymphocytosis excluding three leukaemic cases with T cell counts \(>5.0 \times 10^9/l\) (>5000/mm³).

significantly raised numbers of T cells.
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Table 3 Light chain expression in blood and lymph nodes of NPDLL patients

<table>
<thead>
<tr>
<th>Case No</th>
<th>Blood mononuclear cells</th>
<th>Morphologically abnormal lymphocytes</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k:λ</td>
<td>λ:κ</td>
<td>Immunofluorescence</td>
</tr>
<tr>
<td>Stage I-III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.50</td>
<td>0.67</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>0.66</td>
<td>1.52</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>0.90</td>
<td>0.11</td>
<td>A</td>
</tr>
<tr>
<td>12</td>
<td>0.32</td>
<td>3.08</td>
<td>A</td>
</tr>
<tr>
<td>13</td>
<td>2.57</td>
<td>0.39</td>
<td>A</td>
</tr>
<tr>
<td>39</td>
<td>0.71</td>
<td>1.40</td>
<td>A</td>
</tr>
<tr>
<td>15</td>
<td>3.60</td>
<td>0.28</td>
<td>A</td>
</tr>
<tr>
<td>Stage IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.92</td>
<td>1.09</td>
<td>P</td>
</tr>
<tr>
<td>17</td>
<td>4.09</td>
<td>0.24</td>
<td>A</td>
</tr>
<tr>
<td>20</td>
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<td>23</td>
<td>3.03</td>
<td>0.33</td>
<td>A</td>
</tr>
<tr>
<td>24</td>
<td>0.58</td>
<td>1.72</td>
<td>A</td>
</tr>
<tr>
<td>26</td>
<td>8.60</td>
<td>0.12</td>
<td>A</td>
</tr>
<tr>
<td>27</td>
<td>4.00</td>
<td>0.25</td>
<td>A</td>
</tr>
<tr>
<td>28</td>
<td>1.46</td>
<td>0.68</td>
<td>A</td>
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<td>30</td>
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<td>0.83</td>
<td>P</td>
</tr>
<tr>
<td>33</td>
<td>0.75</td>
<td>0.15</td>
<td>P</td>
</tr>
<tr>
<td>35</td>
<td>0.17</td>
<td>0.05</td>
<td>P</td>
</tr>
<tr>
<td>36</td>
<td>0.17</td>
<td>5.8</td>
<td>P</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;2.75</td>
<td>&lt;1.35</td>
<td>—</td>
</tr>
</tbody>
</table>

Abnormal k:λ or λ:κ ratios are in italic.

P = present.
A = absent.
M = IgM heavy chain; κ = kappa light chain; λ = lambda light chain; — the last two columns in the table illustrate their distribution in lymph nodes as detected by immunofluorescence or immunoperoxidase staining.

Table 4 Immunological findings in the blood of patients with NPDLL at diagnosis and after treatment

<table>
<thead>
<tr>
<th>Case No</th>
<th>Months since diagnosis</th>
<th>wbc (× 10⁹/l)</th>
<th>% lymphocytes</th>
<th>% T</th>
<th>% SLg</th>
<th>% κ</th>
<th>% λ</th>
<th>k:λ</th>
<th>λ:κ</th>
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</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
<td>50</td>
<td>34</td>
<td>41</td>
<td>33</td>
<td>13</td>
<td>40</td>
<td>0.33</td>
<td>3.08</td>
</tr>
<tr>
<td>27</td>
<td>4</td>
<td>4.9</td>
<td>19</td>
<td>74</td>
<td>61</td>
<td>14</td>
<td>42</td>
<td>0.33</td>
<td>2.00</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>6.0</td>
<td>25</td>
<td>33</td>
<td>66</td>
<td>52</td>
<td>13</td>
<td>4-00</td>
<td>0-25</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
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<td>30</td>
<td>8</td>
<td>71</td>
<td>51</td>
<td>35</td>
<td>1-46</td>
<td>0-68</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>8.3</td>
<td>20</td>
<td>13</td>
<td>46</td>
<td>34</td>
<td>14</td>
<td>2-43</td>
<td>0-41</td>
</tr>
<tr>
<td>36</td>
<td>0</td>
<td>14.9</td>
<td>66</td>
<td>15</td>
<td>49</td>
<td>73-5</td>
<td>3-5</td>
<td>21-0</td>
<td>0-05</td>
</tr>
<tr>
<td>36</td>
<td>12</td>
<td>4.9</td>
<td>66</td>
<td>9</td>
<td>64</td>
<td>43-0</td>
<td>13-0</td>
<td>3-3</td>
<td>0-30</td>
</tr>
<tr>
<td>36</td>
<td>12</td>
<td>34.0</td>
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<td>15</td>
<td>58</td>
<td>8</td>
<td>46</td>
<td>0.17</td>
<td>5-8</td>
</tr>
</tbody>
</table>

T cells were identified by rosetting with sheep red blood cells. B cells were identified by immunofluorescence with anti-human IgM (Slg) heavy chain and anti-human kappa (κ) and lambda (λ) light chain antisera.

Abnormal k:λ and λ:κ ratios are in italic.

Discussion

Although abnormalities in the numbers of blood T cells in nodular lymphoma have previously been reported, a correlation between percentage or absolute T cell counts and clinical stage has not been recognised. Our results suggest that blood T cell studies are of value in assessing the extent of disease and may be a useful addition to conventional staging procedures—for example, bone marrow biopsy, lymphangiography. At present the relation between blood T cell counts, prognosis, and response to treatment is unknown but we suggest that reduction of blood T cells may reflect extensive or "bulky" disease. This is of clinical relevance as disease "bulk" appears to be a major prognostic factor in nodular lymphoma.

The finding of an abnormal distribution of immunoglobulin light chains on blood lymphocytes...
in nodular lymphoma is consistent with a previous report by Garrett\(^8\) who found five out of 16 cases of nodular lymphoma with abnormal \(\kappa:\lambda\) ratios. However in Garrett's series only one of 11 cases of NPDLL showed blood abnormalities, the other four cases with abnormal blood \(\kappa:\lambda\) ratios were cases of nodular well-differentiated lymphocytic lymphomas. In the present study 14 of 20 cases of NPDLL were considered to show abnormalities of \(\kappa:\lambda\) ratios indicating an expanded monoclonal B cell population in blood. Abnormalities of light chain expression were found in the absence of either morphologically abnormal cells or lymphocytosis. Surprisingly two cases with lymphocytosis showed normal \(\kappa:\lambda\) ratios although B cells were increased and numerous cleaved cells were present. Failure to detect a monoclonal expansion in these cases was presumably due to adsorbed immunoglobulin on cell membranes, possibly bound by Fc receptors.\(^{15}\)

Immunological studies on lymph nodes were carried out to determine whether abnormalities of immunoglobulin light chain expression on circulating B lymphocytes correspond to that seen in lymph nodes. In 11 cases we were able to demonstrate monoclonal light chain expression in lymph nodes, in the other 9 cases failure to detect monoclonality may have been due to adsorbed surface immunoglobulin or other methodological difficulties in immunofluorescence and immunoperoxidase staining.\(^{16,17}\) In cases in which monoclonality was demonstrated both in lymph nodes and blood the same light chain was abundant in both indicating that cells in blood were of the same clonal origin as neoplastic cells in nodes.

It seems probable that technical difficulties in establishing monoclonality of blood lymphocytes in lymphoma with conventional immunofluorescence procedures leads to an underestimate of cases with monoclonality, and it may be possible to demonstrate a higher incidence of monoclonality in blood of lymphoma patients with more sophisticated techniques such as an fluorescent-activated cell sorter as described by Ligler.\(^9\) Nevertheless, on conventional immunofluorescence microscopy we were able to detect abnormalities in a high proportion of cases of lymphoma, indicating that simple immunofluorescence is still a useful diagnostic procedure.

In five cases in which follow-up blood samples were examined, abnormalities of light chain ratios persisted in four cases despite an apparently satisfactory response to treatment. Although the significance of these findings is at present not known, it is possible that examination of sequential blood samples for evidence of abnormalities of light chains on lymphocytes may provide a method for monitoring the effect of treatment.

We are grateful to the physicians and surgeons of the Edinburgh Lymphoma Group for clinical material used in this study. The work was done under the auspices of the Edinburgh Lymphoma Group in the Department of Pathology, University of Edinburgh. The authors are indebted to Miss EF Ramage, Miss J Kidby and Mr RM Hogg for their expert technical assistance.

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