B-Cell Non-Hodgkin’s Lymphoma: Evidence for the t(14;18) Translocation in All Hematopoietic Cell Lineages

Shai Yarkoni, Michael Lishner, Ilana Tangi, Arnon Nagler, Haya Lorberboum-Galski*

Background: B cells of patients with non-Hodgkin’s lymphoma (B-NHL) harbor specific chromosomal translocations, including t(14;18), the most common aberration found in this disease. The translocation involves the immunoglobulin (Ig) heavy-chain joining (JH) region gene on chromosome 14 and the BCL2 gene on chromosome 18, resulting in dysregulated expression of the BCL2 gene. The t(14;18) translocation has been thought to occur in the pre-B-cell stage, during the first event of Ig gene rearrangement.

Purpose: This study was conducted to investigate the potential involvement of nonlymphoid lineages in B-NHL.

Methods: We studied the t(14;18) translocation and other frequently occurring translocations in total bone marrow aspirates of 10 patients with B-NHL, with the use of the fluorescence in situ hybridization (FISH) technique. We also performed cytogenetic analyses on representative bone marrow aspirates from the patients. Moreover, to define which of the major cell lineages present in the bone marrow carry the t(14;18) translocation, we used a series of monoclonal antibodies together with fluorescence-activated cell sorter (FACS) analyses to purify cells positive for CD3 (T cells), CD19 (B cells), CD10 (CALLA-positive cells), CD41a (megakaryocytic cells), CD13 (myeloid cells), and glycophorin A (erythroid cells). The cells of each subgroup underwent FISH analysis with the use of JH and BCL2 probes to detect the t(14;18) translocation. Bone marrow samples obtained from five healthy donors served as controls.

Results: Bone marrow cells from eight of the 10 patients studied carried the t(14;18) translocation. When present, the translocation was observed in many or even all of the cell lineages (lymphoid, myeloid, megakaryocytic, and erythroid) present in the bone marrow, including peripheral blood progenitor stem cells; for seven of the eight patients carrying the translocation, it was found in 96%-100% of the unfractionated bone marrow cells as well as in all of the FACS-purified cell fractions in which it could be detected or studied. Conventional cytogenetic analyses performed on representative bone marrow aspirates confirmed the results obtained by FISH analysis. Cells in control bone marrow samples obtained from the five healthy donors were negative for the t(14;18) translocation by FISH analysis.

Conclusions: Our findings indicate that the t(14;18) translocation most probably occurs in a very early multilineage progenitor stem cell.

Implications: Given that the t(14;18) chromosomal translocation was found in all types of bone marrow cells when only the B cells were malignant, our results suggest that this translocation is not sufficient to induce neoplastic transformation. This finding underscores the need for the development of new approaches for the detection and surveillance of B-NHL.

B-cell non-Hodgkin’s lymphoma (B-NHL) is considered to originate in a unipotential B-cell progenitor (1). Malignant B-NHL cells are lymphoblasts of lymphocytes with diverse morphologies that characteristically express membrane-associated immunoglobulin (Ig) molecules that function as antigen receptors (2). B-NHL cells are related to bone marrow B cells that have completed V-(D)-J Ig-gene rearrangements but not matured into Ig-secreting plasma cells.

B-NHL cells are known to proliferate in secondary lymphoid organs, such as lymph nodes, spleen- and mucosa-associated lymphoid tissues, and extranodal sites. Lymphoma cells are also found in the peripheral blood and in the bone marrow (3). As in many other hematologic neoplasms (4-6), B-NHL cells harbor specific chromosomal translocations. These involve the Ig loci, including the t(14;18) (q32;q24) translocation, the most common aberration detected in this cancer (7).

Juxtaposition of the BCL2 locus on chromosome 18 with the Ig heavy-chain joining (JH) region gene on chromosome 14 results in dysregulation of BCL2 expression (8,9). The BCL2 protein has been shown to prolong cell survival by delaying programmed cell death (10,11). This chromosomal translocation is believed to be a specific marker of the malignant clone, a hallmark of the disease. It is, therefore, widely used for diagnosis and for monitoring disease progression, minimal residual disease, and response to therapy (12). Other translocations involving the region of the Ig JH and additional proto-oncogenes, such as c-MYC, BCL1, and BCL3 on chromosomes 8, 11, and 19, respectively, are also observed in B-cell cancers, although less frequently. In some hematologic

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See "Notes" section following "References."
cancers, including myelodysplastic syndrome and chronic myelo- 
genous leukemia, a cell committed to a specific lineage pre-
dominates, as in the case of B-NHL. However, these diseases 
are considered to be derived from an early multiprogenitor stem 
cell, and the involvement of other hematologic lineages has also 
been demonstrated (13,14).

Studies (15,16) suggest that multiple myeloma also originates 
in an earlier hematopoietic progenitor than the malignant plasma 
cells and that the bone marrow of such patients contains B-cell 
populations at different stages of differentiation that are clonally 
related to the malignant plasma cells (15,16).

To date, the involvement of other lineages in B-cell cancers 
has not been addressed. We investigated the potential involve-
ment of nonlymphoid lineages in patients with B-NHL by 
analyzing the characteristic chromosomal aberrations found in 
this disease.

Methods

Patients

Bone marrow aspirates (5-15 mL) and peripheral blood leukocytes (PBLs) 
(10-40 mL) were obtained from 10 patients, five at the time of initial diagnosis 
(patients 1, 2, 5, 9, and 10) and five at the time of bone marrow harvesting 
before autologous bone marrow transplantation (patients 3, 4, 6, 7, and 8). All 
subjects were diagnosed as having B-cell NHL by use of morphologic and im-
munohistochemical techniques to examine the PBLs. Written informed 
consent regarding the collection of bone marrow samples for research purposes was ob-
tained from each patient. The investigations conducted for this study were 
approved by a local institutional review board and all institutional guidelines were 
followed in full. Bone marrow and PBL samples were diluted 1:1 (vol/vol) with 
phosphate-buffered saline (PBS), layered onto ficoll—hypaque density gradients 
(Pharmacia LKB Biotechnology AB, Uppsala, Sweden; specific density = 1.077 
g/mL), and centrifuged at 300 g for 30 minutes at room temperature. The low-
density cells were recovered, washed in PBS, and counted. Bone marrow cells 
were processed directly for fluorescence in situ hybridization (FISH) and 
gyogenetic analyses or were further sorted into subgroups (see below). For 
FISH and cytogenetic analyses, cells were incubated with Colcemid (diluted 
1:2000) (Life Technologies, Inc. GIBCO BRL, Gaithersburg, MD) for 90 
minutes at 37°C, treated with 0.5% KCI at 37°C for 30 minutes for hypotonic 
shock, and fixed in methanol:glacial acetic acid (3:1, vol/vol) and dropped onto 
slides precoated with TESPA (3-aminopropyltriethoxysilane, Sigma Chemical 
Co., St. Louis, MO). The slides were kept at 20°C until further analysis.

DNA Probes and Labeling

The JH probe (pHj) used is a 3.5-kilobase (kb) HindIII-EcoRI fragment of the 
human JH gene inserted in a pYT13 plasmid (a 2.9-kb derivative of pBR322); 
the BCL2 probe (pB1-4H) is a 4.2-kb HindIII fragment of the human BCL2 gene 
inserted in a pYT13 plasmid; and the BCL1-b probe (pRH11.3-2) is a 2.1- 
kb Sst1 fragment of the human BCL1 gene cloned in a PUC19 plasmid. The above 
three plasmids were provided by W. Tsujimoto (Wistar Institute, Philadelphia, 
PA). The BCL3 probe, a 1.9-kb EcoRI fragment of the human BCL3 gene 
inserted in a plasmid, was obtained from T. McKeithan (University of Chicago, 
IL). The c-MYC probe is a 8.1-kb HindIII—EcoRI fragment of the human c-MYC 
gene in a pBR322 plasmid (purchased from Oncor, Gaithersburg, MD). All 
probes were subcloned in a bluescript K+ plasmid and labeled with biotin 
(BCL1, BCL2, BCL3, and c-MYC) or digoxigenin (JH), using a nick translation 
protocol (see below) and found to contain no CD13* cells. The CD13* cells 
were detached from the beads by overnight incubation in PBS at 37°C, removed 
with a magnet, and processed for FISH analysis, as described above.

Depletion of CD13-Positive Cells From Bone Marrow 
Aspirates

Bone marrow cells that had been sorted into subgroups (see below) were first 
depleted of CD13-positive (CD13* cells) by incubating the bone marrow cells, 
(2-6 x 10^6 cells) with mouse anti-human CD13 (Silenus Laboratories, Hawthorn, 
VIC, Australia) and anti-mouse immunoglobulin G-coated polystyrene magnetic 
beads (Dynal, M-450, Oslo, Norway), according to the manufacturer’s in-
structions. This procedure was repeated twice to remove the CD13* cells. The 
bone marrow was then re-examined with a fluorescence-activated cell sorter 
(FACS) (see below) and found to contain no CD13* cells. The CD13* cells 
were detached from the beads by overnight incubation in PBS at 37°C, removed 
with a magnet, and processed for FISH analysis.

Flow Cytometry

Bone marrow cells depleted of CD13* cells were treated as follows: 10% of the 
cells were incubated with FITC-conjugated anti-glycoporphin A (Dako, 
Glostrup, Denmark) for detection of erythroid cells; 20% were incubated with 
FITC-conjugated anti-CD3 (Silenus Laboratories) for detection of T cells; 30% 
were incubated with FITC-conjugated anti-CD10 (Silenus Laboratories) for 
detection of CALLA-positive cells; 10% were incubated with FITC-conjugated 
anti-CD19 (Silenus Laboratories) for detection of B cells; and 30% were incu-
bated with FITC-conjugated anti-CD41a (Immunotech S.A., Marseille, 
France) for detection of megakaryocytic cells. CD34-positive (CD34*) cells 
depleted of CD13* cells were separated by flow cytometry, with the use of a 
specific monoclonal antibody (anti-CD34. Oncogene Science, Inc., Cambridge, 
MA).
After a 30-minute incubation at 4 °C, the cells were centrifuged for 10 minutes at 200g at 4 °C. After resuspension in PBS/10% fetal calf serum, 0.02% NaN₃, the cells were kept for up to 12 hours at 4 °C until flow cytometry analysis was performed.

The samples were analyzed with a FACSTAR™ (Becton-Dickinson Immunocytometry Systems, San Jose, CA) using an argon-ion laser tuned to 488 nm. Cells positive for CD3, CD10, CD19, and glycoprotein A (CD3+, CD10-, CD19+, and glycoprotein A+ cells, respectively) cells were sorted out and collected with the use of high-fluorescence, low-side-scatter gating; the CD41-positive (CD41+) cells were sorted out, using high-fluorescence, high-forward-scatter gating. Mature platelets and erythrocytes were gated out using a threshold of 100.

On reanalysis of the recovered CD10+ and CD41+ cells, more than 90% were found to be positive for the particular selection marker, as were more than 96% of the CD3+, CD19+, and glycoprotein A+ cells. Sorted cells were centrifuged at 300g for 7 minutes at room temperature (i.e., cytocentrifuged) on slides, stained with hematoxylin-eosin, and examined morphologically. The cell specimens were found to be more than 95% homogeneous.

The bone marrow-sorted subgroups were treated with 0.5% KCl for 30 minutes at 37 °C, fixed in methanol:acetic acid (3:1, vol/vol), and dropped onto precoated (TESPA) slides for FISH analysis.

**Results**

The t(14;18) translocation and other frequently occurring translocations involving chromosome 14 (JH) and the proto-oncogenes c-MYC, BCL1, and BCL3 (located on chromosomes 8, 11, and 19, respectively) were studied in 10 patients with B-NHL, with the use of the sensitive FISH technique.

The various translocations were examined in bone marrow aspirates that were processed for FISH and cytogenetic analysis immediately after sampling. The clinical characteristics of the patients studied are summarized in Table 1.

As shown in Table 2, eight (80%) of 10 bone marrow samples carried the t(14;18) translocation. Fig. 1, A shows the results of a FISH analysis performed on the bone marrow of patient 1, with the use of JH and BCL2 as probes. Two patients (Nos. 7 and 9) had no detectable translocations of JH with any of the proto-oncogenes tested (Tables 2 and 3).

In seven of eight patients (excluding patient 6; Table 1) who carried the t(14;18) translocation, 96%-100% of the screened bone marrow cells exhibited the chromosomal aberration (Table 3). No or extremely few (<3%) cells with two distinct sets of signals representing separate alleles were found in the bone marrow of these patients. The only exception was patient 6 in whom the t(14;18) translocation appeared in only approximately 50% of the bone marrow cells (Table 3). One patient (No. 10; Table 2) displayed the t(14;18) translocation and an additional translocation t(14;19), involving chromosome 14, in 50% of the bone marrow cells evaluated.

The results of the conventional cytogenetic analysis performed on five bone marrow aspirates (patients 2, 5, 7, 8, and 9) were in full agreement with the results obtained by FISH analysis (Table 2). In patients 2 and 8, cytogenetic analysis revealed additional translocations: t(8;21) and t(3;10), respectively (Table 2).

In the bone marrow aspirates from the five healthy donors, no or extremely few (<3%) cells carried the t(14;18) translocation (Table 2; Fig. 1, C).

In a previous study (17), the t(14;18) translocation was detected in 30%-50% of the patients with diffuse B-NHL and in 85% of patients with follicular lymphoma. The t(14;18) translocation was presented in seven (78%) of nine of our patients with diffuse B-NHL. This discrepancy may reflect the various

### Table 1. Pathologic characteristics of patients with B-cell non-Hodgkin's lymphoma

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pathologic diagnosis*</th>
<th>Involvement of bone marrow†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Follicular mixed-cell lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Diffuse large-cell lymphoma</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Diffuse large-cell lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse large-cell lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Diffuse large-cell lymphoma</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Diffuse large-cell lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Large-cell immunoblastic lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Large-cell immunoblastic lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Small non-cleaved-cell lymphoma</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Small non-cleaved-cell lymphoma</td>
<td>+</td>
</tr>
</tbody>
</table>

*Pathologic diagnosis and lymphoma classification were based on the working formulation of non-Hodgkin's lymphoma for clinical use (34).
†Bone marrow involvement was assessed morphologically by an oncologist.

### Table 2. Translocations in bone marrow cells of patients with B-cell non-Hodgkin's lymphoma

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Translocation*</th>
<th>Cytogenetic analysis†</th>
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<tbody>
<tr>
<td>1</td>
<td>JH/BCL1 t(14;18)</td>
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</tr>
<tr>
<td>2</td>
<td>JH/BCL1 t(14;11)</td>
<td>Not determined</td>
</tr>
<tr>
<td>3</td>
<td>JH/BCL3 t(14;19)</td>
<td>Not determined</td>
</tr>
<tr>
<td>4</td>
<td>JH/c-MYC t(14;8)</td>
<td>Not determined</td>
</tr>
<tr>
<td>5</td>
<td>JH/BCL1 t(14;11)</td>
<td>Not determined</td>
</tr>
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<td>6</td>
<td>JH/BCL3 t(14;19)</td>
<td>Not determined</td>
</tr>
<tr>
<td>7</td>
<td>JH/c-MYC t(14;8)</td>
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</tr>
<tr>
<td>8</td>
<td>JH/BCL3 t(14;19)</td>
<td>Not determined</td>
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<tr>
<td>9</td>
<td>JH/c-MYC t(14;8)</td>
<td>Not determined</td>
</tr>
<tr>
<td>10</td>
<td>JH/c-MYC t(14;8)</td>
<td>Not determined</td>
</tr>
<tr>
<td>Control subject† (n = 5)</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

*The JH/BCL1, BCL2, BCL3, and c-MYC translocations were determined by fluorescence in situ hybridization analysis. In each case, 50-100 cells were screened for each translocation.
†Cytogenetic analysis was performed using the Giemsa-staining technique. Ten to 20 metaphases were analyzed for each patient.
‡All five control subjects were negative for all the translocations tested. - = no translocation detected; + = translocation present.
Table 3. The t(14;18) translocation in various hematologic cell lineages of patients with B-cell non-Hodgkin's lymphoma*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bone marrow</th>
<th>CD3</th>
<th>CD10</th>
<th>CD19</th>
<th>CD13</th>
<th>CD41a</th>
<th>Glycophorin A</th>
<th>CD34†</th>
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<tr>
<td>1</td>
<td>64/66</td>
<td>32/33</td>
<td>16/16</td>
<td>64/67</td>
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<td>62/63</td>
<td>18/19</td>
</tr>
<tr>
<td>2</td>
<td>97/98</td>
<td>28/29</td>
<td>NA</td>
<td>41/42</td>
<td>32/32</td>
<td>11/11</td>
<td>41/42</td>
<td>NA</td>
</tr>
<tr>
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<td>NA</td>
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<tr>
<td>4</td>
<td>78/79</td>
<td>47/48</td>
<td>NA</td>
<td>34/35</td>
<td>112/114</td>
<td>6/7</td>
<td>94/95</td>
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<td>94/96</td>
<td>26/26</td>
<td>32/32</td>
<td>13/13</td>
<td>NA</td>
<td>22/23</td>
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<td>46/47</td>
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<td>54/55</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>84/86</td>
<td>28/30</td>
<td>9/9</td>
<td>24/24</td>
<td>NA</td>
<td>7/7</td>
<td>43/43</td>
<td>NA</td>
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<td>9</td>
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<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>71/72</td>
<td>42/44</td>
<td>NA</td>
<td>39/40</td>
<td>42/44</td>
<td>12/13</td>
<td>43/45</td>
<td>NA</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.976</td>
<td>0.972</td>
<td>0.956</td>
<td>0.979</td>
<td>0.974</td>
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<tr>
<td>C.L.</td>
<td>0.969, 0.993</td>
<td>0.957, 0.987</td>
<td>1.00</td>
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<td>0.959, 0.985</td>
<td>0.910, 1.002</td>
<td>0.965, 0.993</td>
<td></td>
</tr>
</tbody>
</table>

*All translocations were determined by fluorescence in situ hybridization analysis. The results are expressed as the number of positive cells carrying the translocation out of the total number of cells screened. - = no t(14;18) translocation detected. NA = not available.
†CD34-positive progenitor cells (CD34⁺/CD13⁻) were obtained from peripheral blood. C.L. = confidence limits (95%).
‡This mean was calculated without including patient number 6.

Fig. 1. Fluorescence in situ hybridization (FISH) analysis of unfractonated bone marrow cells (A) and of CD34-positive (CD34⁺) cells (B) from a patient with B-NHL, demonstrating the t(14;18) translocation. (C) Control cells lacking the t(14;18) translocation. FISH analysis was performed using the JH and BCL2 probes. The translocation is indicated by the yellow–orange spot; the two alleles JH and BCL2, by the green spot and red–orange spot, respectively.
techniques used and the number of cells in metaphase in the samples studied, as well as variations in the sensitivity of the methods.

After determining which patients carried the t(14;18) translocation, we attempted to define which of the major cell lineages in their bone marrow carried the t(14;18) translocation. The bone marrow of each patient carrying the translocation was depleted of CD13+ cells with the aid of magnetic beads. Flow cytometry with the use of a series of monoclonal antibodies was then used to characterize and sort out CD3+, CD19+, CD10+, CD41a+, and glycophorin A+ cells. The CD13+ cells were separated from the magnetic beads and reanalyzed by FACS.

After sorting the various bone marrow subgroups, FACS analysis and morphologic assessment revealed over 95% purity. Fig. 2 demonstrates the sorting of the CD3+, CD19+, and glycophorin A+ cells from a representative patient with B-NHL.

All subgroups were processed for FISH analysis, with the use of the JH and BCL2 probes as described above. In seven of the eight patients carrying t(14;18) in their unfractionated bone marrow (Table 2), the translocation was found in 96%-100% of the presorted subgroups representing the main bone marrow cell lineages (Table 3). These included the lymphoid cell subsets T (CD3+), B (CD19+), and CALLA-positive cells (CD10+), as well as the myeloid (CD13+), megakaryocytic (CD41a+), and erythroid (glycophorin A+) hematopoietic cell lineages. Thus, the t(14;18) translocation was found in almost every cell screened by FISH, independent of its clonal origin (Table 3). The ratio of t(14;18)-negative cells in each cell subgroup was less than 3%. In one patient (No. 6), the t(14;18) translocation was found only in the lymphoid CD3+ and CD19+ cells, while the other cell lineages (myeloid, megakaryocytic, and erythroid) proved to be negative for this translocation (Table 3).

Since all cell lineages were positive for the t(14;18) translocation in seven of the eight patients, we assumed that the hematopoietic progenitor cells also carried the t(14;18) translocation. We therefore separated the CD34+ progenitor cells from the CD13+-depleted peripheral blood cells of two patients (Nos. 1 and 4; Table 2) with the use of monoclonal antibody anti-CD34. Cells were separated to more than 85% purity by FACS (double sorting). As shown in Fig. 1, B and Table 3, in both patients the hematopoietic progenitor cells (CD34+/CD13−) carry the t(14;18) translocation. As in all the other subgroups screened, the t(14;18) translocation was detected in 96%-100% of the cells examined (Table 3).

**Discussion**

The search for the cell of origin from which a malignant clone has developed has always been one of the fields of interest in cancer research. Tracing the early precursors can shed light on the pathophysiology of a particular disease. Furthermore, it can lead to the development of new strategies for treatment and surveillance.

To evaluate the involvement of cell lineages other than the malignant clone in B-NHL, we studied the t(14;18) translocation in total bone marrow and presorted cell subgroups of patients with B-NHL with the use of the FISH technique.

Our results clearly demonstrate that in most patients with B-NHL, all of the cell lineages present in the bone marrow, including peripheral blood CD34+ progenitor stem cells, carry the
t(14;18) translocation. This led us to conclude that this translocation occurs in a very early multilineage progenitor cell.

Our findings contradict a previous report (18) suggesting that the t(14;18) translocation occurs in the pre-B-cell stage during the first event in Ig-gene rearrangement because of an error of the V-(D)-J recombinase. The latter premise was based on the assumption that the recombinase is the sole enzyme involved in this event, which occurs exclusively in B cells. However, the stage at which the V-(D)-J recombinase first appears has not been rigorously identified. Moreover, there are reports to the effect that the expression of V-(D)-J recombinase is not limited to lymphoid cells and that the enzyme is active in myeloid cell-lineage tumors (19) and even in the brain (20). These observations lend support to our findings.

In addition, it is not necessary to assume that all chromosomal translocations are mediated by recombinases that normally mediate antigen-receptor gene rearrangements. There are other enzymatic systems that are involved in genetic recombination, such as those that mediate the excision and reintroduction of viral sequences and mobile genetic elements, including transposomes. The possible role of such recombinases in cellular chromosomal translocations characteristic of neoplasia is largely unexplored.

It is commonly believed that B-NHL cells are clonal disorders of a mature-stage B cell that have a normal counterpart in the B-cell lineage. In this article, we demonstrate the existence of the t(14;18) translocation in all hematopoietic cell lineages, even those not considered to be part of the malignant clone. Our results may, therefore, suggest that a cell carrying the t(14;18) translocation is itself most probably not sufficient to induce tumorigenicity. Several lines of evidence support our findings that the t(14;18) translocation does not render the cell tumorigenic. Transfection of BCL2 into cells or immortalized B cells fails to influence the tumorigenicity of the cells, which are inefficient in forming tumors when injected into athymic mice (21,22). However, when BCL2 was overexpressed, together with c-MYC, a high incidence and a short latency of tumor formation was noted. Limpens et al. (23) detected the t(14;18) translocation in non-neoplastic human tonsil tissue. Also, in a remarkably large number of cases, evidence of two different t(14;18) translocations was found in the same patient (24). This implies that the t(14;18)-transforming event may occur far more frequently than is reflected by the incidence of lymphomas. The implication of these findings may be that most of the potentially malignant t(14;18)-carrying cells generated in the course of a lifetime are eliminated either by the immune system or by other mechanisms or else never progress to tumors. Indeed, this supposition finds support in a recent finding that clones harboring the t(14;18) translocation are commonly present in the peripheral blood and spleen of normal individuals and that the frequency of the translocation as well as the probability of developing lymphoma increase significantly with age (25).

The response of follicular NHL to therapy further confirms this finding, since successful response to treatment and cure results in the complete elimination of the fully transformed malignant clone, while clones containing less than the full complement of genetic abnormalities required for neoplasms remain. It was reported that cells bearing the t(14;18) translocation (detected by the polymerase chain reaction) are present in long-term (10-15 years) survivors of localized follicular lymphoma (26). Such cells either represent "dormant" lymphoma cells, possibly awaiting activation, or more likely, belong to a pre-malignant clone that turns malignant only with the accumulation of additional genetic abnormalities.

Anemia and/or thrombocytopenia in untreated B-NHL has been previously described (27). Moreover, biphenotypic hematologic cancers involving both lymphoid and myeloid phenotypic markers have been reported (28). Several patients with NHL who underwent autologous stem cell transplantation developed myelodysplastic syndrome or acute myeloid leukemia after transplantation (29), possibly indicating the common origin of the two diseases.

High-dose chemotherapy followed by autologous stem cell transplantation is an accepted mode of therapy in patients with B-NHL and results in long-term, disease-free survival (30). However, the relapse rate remains high and poses a major obstacle to autologous stem cell transplantation. Our finding that the t(14;18) translocation is an early progenitor cell event and that it is detectable in all hematopoietic cell lineages may provide an additional explanation for the high relapse rate.

It is well established that most human tumor clones arise from a single transformed cell and that tumor progression is a multi-step process requiring multiple mutations in oncogenes and tumor-suppressor genes (31-33). The finding that, in three of eight patients with B-NHL carrying the t(14;18) translocation, an additional aberration (translocation) was detected (Table 2) is in accordance with this theory. It is interesting that, in patient 10, the percentage of cells (50%) bearing the additional t(14;19) translocation (as determined by FISH analysis) appeared to be correlated with the percentage of malignant cells found in the bone marrow (as determined by morphologic and immunohistochemical assessment) (results not shown). These clinical reports, together with our results demonstrating that the t(14;18) translocation is found in all bone marrow cells, while only the B cells are malignant, point to the need for additional research to elucidate how a cell bearing the t(14;18) translocation is transformed into a malignant cell. Our results also imply that following the occurrence of a t(14;18) translocation, even the use of the most advanced molecular techniques cannot predict either an cure or the persistence of the malignant disease. This must await in-depth knowledge of the molecular mechanisms involved in B-cell malignant transformation.

References


Notes

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