The presence of the translocation t(8;14)(q24;q32) has not been well described in follicular lymphoma (FL). In a consecutive series of 278 karyotypically abnormal non-Hodgkin's lymphomas (NHL), six patients with FL showing a t(8;14) without a t(14;18)(q32;q21) were identified. They ranged in age from 45 to 73 years. The cell type was mixed in four patients, small-cleaved in one, and large-cleaved in one; four cases also contained diffuse areas. All cases tested displayed monoclonal surface Ig. The clinical courses were consistent with the histologic subtypes, being less aggressive than other t(8;14)-bearing NHL. In five cases, frozen tissue was available for Southern blotting. The BCL2 gene showed a germine configuration when studied with the MBR, MCR, and 5' cDNA probes. The MYC gene also appeared unarranged using an exon-1 probe with EcoRI or HindIII digestion. Analysis of the Ig heavy chain (IGH) gene with a JH region probe and BamHI or EcoRI digestion showed only one rearranged band in all cases, indicating that the 14q32 breakpoint did not lie in either the J or switch-mu (SM) regions. In four cases, the exon-1/intron-1 border of the MYC gene, a target area for point mutations in cases of t(8;14) that do not display rearrangements of the MYC gene, was enzymatically amplified and sequenced; no point mutations were identified. The indolent behavior of our six cases, and the finding that the molecular structure of the t(8;14) in these cases does not follow the pattern of breakpoint sites and point mutations defined in other histologic subtypes of NHL with this translocation, suggest that the t(8;14) in these cases is cytogenetically and molecularly distinct from the t(8;14) seen in high-grade NHLs, and is relatively ineffectual in terms of MYC deregulation, or that other genetic elements at these chromosomal sites may be involved. Further analysis of these tumors may provide insights into MYC deregulation and BCL2-independent FL.

Approximately 70% of cases of follicular lymphoma (FL) are characterized by the recurrent translocation t(14;18)(q32;q21), which juxtaposes the IGH gene at 14q32 and the BCL2 oncogene at 18q21. This translocation can also be shown at the molecular level by Southern blotting using probes to the various regions; however, the proportion of positive cases is essentially the same as in groups of cases with informative karyotypes. Hence, a substantial minority of FL cases, roughly 30%, do not seem to arise by translocation-mediated BCL2 deregulation. The cytogenetic and molecular features of this subset of FL are still poorly understood. We report here six cases of FL with the t(8;14)(q24;q32) in the absence of the t(14;18). This unusual histologic-cytogenetic constellation has not been previously analyzed at the molecular level. The t(8;14) is almost exclusively seen in diffuse non-Hodgkin's lymphoma (NHL), typically Burkitt's lymphoma (BL). This translocation brings the MYC oncogene at 8q24 under the control of IGH gene at 14q32. Our present study of the configuration of the MYC and BCL2 genes in this subset of FL with t(8;14) suggests that the t(8;14) in these cases may be a cytogenetically and molecularly distinct variant of the classic translocation.

**Material and Methods**

The six cases reported here are taken from a consecutive series of 434 NHLs received for cytogenetic analysis at the Memorial Sloan-Kettering Cancer Center from January 1984 to December 1988. Abnormal karyotypes were obtained on a total of 278 cases. The proportions of informative cases and the patterns of different abnormalities among the various histopathologic subtypes of NHL are presented in detail elsewhere. Briefly, the series included 69 NHL with abnormal karyotypes, of which 50 showed the t(14;18) (72%), and 41 NHL with the t(8;14), seven of which were follicular. One of these seven cases also had a translocation involving 18q21, and was therefore excluded from this report.

The patients were staged according to the Ann Arbor system and their lymphomas were classified pathologically according to the International Working Formulation. The cell surface immunophenotype determination and the cytogenetic analysis, which consisted of short-term culture followed by Q-banding, were performed as previously described. The karyotypes are reported according to the ISCN nomenclature. The clinical follow-up was obtained up to December 1990. Survival analysis was performed on subsets of patients with differing cytogenetic characteristics using the method of Kaplan and Meier. Actuarial survival curves were compared using the logrank test.

Gene rearrangements were studied by Southern blotting of DNA extracted from snap-frozen tissue, using an Oncor Probe-Tech vacuum blotting apparatus (Gaithersburg, MD). The following combinations of probes and enzymes were used. For the IGH gene, a 5.5-kb fragment encompassing the entire JH region was used with EcoRI- or BamHI-digested DNA, to provide information on the configuration of the SM region as well. For the BCL2 gene, DNA digested with BamHI, EcoRI, Pst I, or HindIII was studied with three different probes: a 4.3-kb HindIII fragment of the major breakpoint region (MBR), a 4-kb fragment of the minor
cluster region (MCR), and a 1.6-kb EcoRI cDNA fragment of the second exon (5' cDNA). For the MYC gene, DNA digested with EcoRI or HindIII was probed with the Xho I-Xba I fragment of the first exon.

Sequencing of the exon-1/intron-1 region of the MYC gene was carried out following a previously described protocol based on the polymerase chain reaction (PCR). The PCR was performed on 1 μg of genomic DNA extracted from frozen tissue, in 10 mmol/L of each deoxynucleotide, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl₂, 0.01% gelatin, with 20 pmol of each primer. Two sets of primers were used: primers 763 and 764, corresponding, respectively, to positions 2716 to 2735 and 2872 to 2891 of the sequence of the MYC gene, and, similarly, primers 960 and 961, from positions 2803 to 2822 and 3041 to 3061. The temperature cycling parameters were as follows: denaturing at 95°C for 15 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 90 seconds, for 25 cycles with a final extension step of 7 minutes. The reaction product was then gel-purified in low melting-point agarose and sequenced according to a modification of the protocol of McMahon et al.

RESULTS

Clinical data. The salient clinical features are summarized in Table 1. The patients' age at the time of study ranged from 45 to 73 years. The median age of the FLs with the t(8;14) (58 years) was not different from the mean age of FLs with karyotypic abnormalities other than t(8;14) (P > .05).

Four patients (cases 520, 612, 682, and 697) presented with stage II disease, and two patients (cases 312 and 691) presented with more extensive disease, including marrow involvement. The clinical courses of these six patients were similar to other FLs with abnormal karyotypes; there was no significant difference in overall survival between the two groups (median survival not reached, P > .05). All six patients were alive at a median follow-up of 42.5 months from diagnosis. The three patients (cases 312, 612, and 697) in complete remission had all received anthracycline-containing combination chemotherapy; cases 312 and 612 at the time of relapse, and case 697 at the time of presentation. Case 312 had received no treatment for 4 years after diagnosis; adenopathy progressed and responded to CHOP chemotherapy. Three patients (cases 520, 682, and 691) had relapsing disease despite multiple trials of non-anthracycline-containing regimens. Patient 682 received no treatment for 2 years after diagnosis, and had 2 years of palliative therapy with α-interferon and alkylating agent chemotherapy. A repeat biopsy at the most recent follow-up visit showed persistent follicular mixed lymphoma.

Histopathology and immunophenotype. The histologic features of cases 312, 520, 682, and 697 are shown in Fig 1. The cell types of the lymphomas were small-cleaved cell in case 682, large-cleaved cell in case 697, and mixed in the remaining four cases. Cases 312, 520, 612, and 691 also

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Path</th>
<th>Surface Ig</th>
<th>Genes</th>
<th>Biopsy</th>
<th>Stage</th>
<th>Therapy</th>
<th>Survival</th>
<th>Karyotype</th>
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<tbody>
<tr>
<td>312</td>
<td>70/M</td>
<td>FM</td>
<td>IgM-κ</td>
<td>-</td>
<td>MYC, BCL2</td>
<td>DX</td>
<td>IV</td>
<td>None</td>
<td>CHOP</td>
<td>NED, 40+ mo</td>
</tr>
<tr>
<td>520</td>
<td>64/F</td>
<td>FM</td>
<td>IgM-κ</td>
<td>G</td>
<td>NHL4, Mitox Cycl Vcr Dec</td>
<td>NHL4, MACOPB</td>
<td>AWD, 38++ mo</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>612</td>
<td>59/F</td>
<td>FM</td>
<td>IgG-κ</td>
<td>G</td>
<td>NHL4</td>
<td>REL</td>
<td>II</td>
<td>MACOPB</td>
<td>NED, 85+ mo</td>
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<tr>
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<td>73/F</td>
<td>FSC</td>
<td>-</td>
<td>G</td>
<td>REL</td>
<td>CVP</td>
<td>II</td>
<td>VGPL</td>
<td>AWD, 198+ mo</td>
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<tr>
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<td>G</td>
<td>REL</td>
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<td>IFN</td>
<td>CMOPP</td>
<td>AWD, 45+ mo</td>
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<tr>
<td>697</td>
<td>58/F</td>
<td>FLC</td>
<td>IgM-κ</td>
<td>G</td>
<td>DX</td>
<td>NHL9</td>
<td>None</td>
<td>None</td>
<td>NED, 29+ mo</td>
<td>31</td>
</tr>
</tbody>
</table>

Abbreviations: FM, follicular mixed; FLC, follicular large cell; FSC, follicular small cleaved; -, not available; G, germline; R, rearranged; DX, diagnosis; REL, relapse; NHL4, thiotepa + vincristine + chlorambucil + prednisone; IFN, α-interferon; CHOP, cyclophosphamide + doxorubicin + vincristine + prednisone; MACOPB/NHL9, methotrexate + doxorubicin + cyclophosphamide + vincristine + prednisone + bleomycin; Mitox, mitoxantrone; Cycl, cyclophosphamide; Vcr, vincristine; Dec, decadron; Gallium, Gallium nitrate; m-GAG, methyl GAG; MoAb, monoclonal antibody; CMOPP, cyclophosphamide + vincristine + procarbazine + prednisone; CVP, cyclophosphamide + vincristine + prednisone; AWD, alive with disease; NED, alive with no evidence of disease.

*No chemotherapy before cytogenetic analysis (patient also had a previously resected renal cell carcinoma).
showed focal diffuse areas. In none of the cases was a component of BL observed. All cases studied for surface Ig showed a monoclonal pattern consisting of IgM in four and IgG in one.

**Cytogenetics.** The specimens for chromosome analysis were obtained before therapy in cases 312, 520, and 697. Patients 612, 682, and 691 were studied in relapse. Partial karyotypes of cases 520, 682, and 697 are shown in Fig 2. Visually, the breaks in 8q24 as well as 14q32 appeared to be slightly distal to those normally seen in the t(8;14) of BL and diffuse large cell lymphoma, suggesting that this may be a subset of t(8;14) translocations that are different from those encountered in high-grade lymphomas. In addition to the t(8;14), other karyotypic changes were present in each case (Table 1). Four patients (cases 312, 520, 691, and 697) had trisomy 3. Five had abnormalities of chromosome 1, but with different breakpoints: cases 520 and 682 at band 1q21, and cases 612 and 697 at band 1p36. Three cases showed involvement of 6q, which in cases 520 and 612 took the form of a t(1;6) with a breakpoint at 6q23. Cases 691 and 697 also showed trisomy 18.

These six cases of FL with the t(8;14) accounted for 9% of the FL with abnormal karyotypes, and 22% of the NHL with the t(8;14) in our consecutive series of NHL with cytogenetic data.³

**Gene rearrangement.** DNA was available for Southern blot analysis in all cases except case 312. Probing of BamHI-digested genomic DNA with a JH probe showed in each case one rearranged band and one germline band (Fig 3), confirming the presence of tumor DNA in the DNA samples studied. Southern analysis of the MYC gene showed only germline bands (Fig 3). Probing of the BCL2 region with the MBR, MCR, and 5' cDNA probes also showed a germline configuration in all cases.

**PCR sequencing.** The exon-1/intron-1 region of the MYC gene was sequenced using a PCR-sequencing approach in cases 520, 612, 691, and 697. No point mutations or other minor alterations were detected.

**DISCUSSION**

Studies correlating specific translocations with histopathologic subtypes of NHL have established that approximately 70% of FLs contain a t(14;18), detectable either at the cytogenetic or molecular level.⁴,⁵,⁶ Aside from one case of small-cleaved cell FL included in the Fifth International Workshop on Chromosomes in Leukemia-Lymphoma,² there are no reported cases of FL with the t(8;14) without the t(14;18).

Because of the small size of the present study group, it was not possible to discern a distinctive pattern of clinical
expression. It seems clear, however, that the presence of the 
\( t(8;14) \) does not connote a more aggressive behavior for this 
subset; all six patients were alive at a median follow-up of 
over 3 years, despite a variety of treatment approaches.

Interestingly, all three patients treated with “aggressive” 
anthracycline-containing regimens, including two patients 
in relapse, had complete responses. However, the follow-up 
of these patients is short.

Four of our six cases of FL showed diffuse areas. The 
clinical significance of diffuse areas remains controversial: 
some studies have found a negative impact on prognosis in 
the mixed and large cell categories,\(^{21,22}\) which others have 
been unable to corroborate.\(^{23}\) A large cell component was 
present in all cases except case 682, and was predominant in 
case 697. Among the subtypes of FL, the large cell type 
\( t(8;14) \) (q24;q32) is shown in 
each case. The breakpoints are 
indicated by arrows. Case 697 
had trisomy 18, although the 
chromosomes 18 appear structurally normal in the three cases 
illustrated.

Fig 2. Q-banded partial karyotypes of cases 520, 682, and 697. 
The \( t(8;14)(q24;q32) \) is shown in 
each case. The breakpoints are 
indicated by arrows. Case 697 
had trisomy 18, although the 
chromosomes 18 appear structurally normal in the three cases 
illustrated.

Fig 3. Southern blot analysis of the \( I gH \) gene (top) and of the \( M Y C \) 
gene (bottom) in cases 520, 612, 682, and 691. The genomic DNAs in 
this figure were digested with \( B a mH I \) for analysis of the \( I gH \) gene, and 
with \( E c o R I \) for analysis of the \( M Y C \) gene, except in case 682, in which 
\( H i n d I I I \)-digested DNA was used for the latter. The arrowheads indi-
cate the position of the expected germline bands. In each case, a 
single rearranged \( I gH \) band is observed, whereas no rearrangements 
are seen in the analysis of the \( M Y C \) gene. (The germline \( E c o R I \) and 
\( H i n d I I I \) \( M Y C \) bands have been aligned merely for illustrative purposes.)

Other recurrent karyotypic abnormalities seen in our 
cases included trisomy 3 and trisomy 18, as well as structural 
abnormalities of chromosomes 1 and 6. We have 
previously demonstrated a significant correlation between 
trisomy 3 and trisomy 18.\(^1\) Structural aberrations involving 
1p36, 1q21, and 6q are common aberrations in lymphoid 
neoplasms with nonspecific histopathologic correlates, but 
have been shown to be negative prognostic factors in diffuse 
NHL with a large cell component.\(^{1,24}\) The occurrence of 
trisomy 3 and 6q abnormalities in FL has been correlated in 
some studies with the histologically more advanced sub-
types of low-grade NHL.\(^{1,25}\)

The clinicopathologic presentation in our series of cases 
differs markedly from cases of \( t(14;18) \)-positive FL, in 
which a \( t(8;14) \) or variant occurs as an additional aberration. 
Cases of NHL with concurrent \( t(8;14) \) or variants and 
\( t(14;18) \) are typically high-grade NHL or acute lymphoblasto-
lastic leukemia.\(^{26,27}\) In the two cases that have been most 
extensively analyzed,\(^{28,29}\) various lines of evidence indicated that 
the \( t(8;14) \) was the secondary change responsible for 
the transformation of a \( t(14;18) \)-bearing FL. This pathway 
of lymphoma progression has also recently been demonstrated 
experimentally in a transgenic mouse model.\(^30\) We
are aware of only one published case of NHL with both MYC and BCL2 rearrangements in which the follicular histology was maintained (case 3 in De Jong et al.26). In our own series of NHL, we have found three cases with both translocations, in either their classic or variant forms: one small-cleaved cell FL with diffuse areas, one BL21 and one large-cell immunoblastic lymphoma (case 143 in Ladanyi et al1). A common feature of lymphoid neoplasms bearing both translocations is the absence of Ig heavy chain expression,22,27 caused by the involvement of both IgH alleles by translocations. In contrast, our cases of FL with the t(8;14) demonstrated monoclonal surface Ig.

In sporadic BL and acquired immunodeficiency syndrome (AIDS)-associated BL with t(8;14), the switch regions of the IgH gene and sequences in the first intron, first exon, or immediately 5' are involved; whereas in endemic BL with t(8;14), the translocation breakpoints usually involve JH region, at 14q32, and poorly characterized sequences at least 90 kb upstream of the MYC gene.22,23. In all variant translocations, the breakpoints are located at a considerable distance downstream of the MYC gene, where, in some cases, they appear to involve the newly described PVT transcriptional unit, which starts 57 kb downstream of MYC.34 The role of the human PVT gene is unknown, but its regulatory regions (unpublished observations, 1990). Therefore, we analyzed the exon-l/intron-1 region of the MYC gene. One of the principal molecular biologic correlates of the breakpoint location in BL is the occurrence of point mutations in the 5' regulatory regions of the MYC gene. Most cases of BL with 8q24 breakpoints outside of the MYC gene show such mutations at the site of a partial transcriptional block at the 3' end of exon-134,39; most, if not all, of the remaining cases show point mutations elsewhere in the 5' regulatory regions (unpublished observations, 1990). Therefore, we analyzed the exon-1/intron-1 region of the MYC gene by a PCR-sequencing approach, to determine if the same correlation as in BL existed in the present series of cases. None of the four cases examined showed any mutations in this portion of the MYC gene.

Because all cases tested displayed monoclonal surface Ig, the single rearranged band on the Southern analysis of the JH region must belong to the productively rearranged allele. Hence, at least in these cases, the breakpoint at 14q32 does not lie in the JH or the SM regions. Occasionally, t(8;14) breakpoints occur in germline switch-α or switch-γ regions,34,43 which we did not study. Breakpoints involving band 14q32 outside of the IgH gene altogether have also been reported in some lymphoid neoplasms.40,43

A germline configuration of the BCL2 gene was demonstrated with the MBR, MCR, and 5' cDNA probes in our five cases with available DNA. In about 30% of FL, these probes failed to detect a rearrangement.3. Interestingly, a comparable subset of FL also show no immunohistochemical evidence of BCL2 overproduction.44,45 The subset of FL lacking BCL2 rearrangement has not been studied in detail. Yunis et al46 found the absence of a BCL2 rearrangement to have a positive impact on prognosis in FL of mixed- or large-cell type; however, a similar relationship has not been demonstrated in studies containing all subtypes of FL.47,48 Most cases of FL lacking the t(14;18) are of the mixed- or large-cell type. 1.3 Interestingly, such cases commonly bear additional chromosome 18 material, as in our cases 691 and 697, which both show trisomy 18, and this has been correlated, in a small series, with BCL2 protein overexpression.49

Given that both the chromosome 8 and chromosome 14 breakpoints in the present cases seem to fall outside of the commonly affected sites, the t(8;14) in these cases is molecularly distinct from the t(8;14) encountered in high-grade NHL and may be relatively ineffectual in terms of MYC deregulation, which would be consistent with the indolent clinical behavior observed in our cases, which is more akin to low-grade NHL than to other t(8;14)-bearing NHL. Alternatively, the t(8;14) in these cases may involve altogether different, as yet undefined, genetic elements at 8q24 and 14q32, which is consistent with the cytogenetic observation that the breakpoints in these translocations may be slightly distal to those seen in the t(8;14) associated with high-grade NHL.

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