Molecular Genetics of Gastrointestinal Non-Hodgkin's Lymphomas: Unusual Prevalence and Pattern of c-myc Rearrangements in Aggressive Lymphomas

By J.H.J.M. van Krieken, M. Raaffeld, S. Raghoeber, E.S. Jaffe, G.J.B. van Ommen, and Ph.M. Kluin

Thirty-two extranodal lymphomas of the gastrointestinal (GI) tract underwent molecular genetic analysis by Southern blotting using probes for the immunoglobulin genes and the bcl-1, bcl-2, and c-myc loci, commonly involved in lymphomagenesis. No bcl-1 rearrangements were found. There was only one large-cell lymphoma with a bcl-2 rearrangement. A rearrangement of the c-myc gene was found in six of eight Burkittlike lymphomas of the intestine. Thirty-two extranodal lymphomas of the gastrointestinal tract underwent molecular genetic analysis by Southern blotting using probes for the immunoglobulin genes and the bcl-1, bcl-2, and c-myc loci, commonly involved in lymphomagenesis. No bcl-1 rearrangements were found. There was only one large-cell lymphoma with a bcl-2 rearrangement. A rearrangement of the c-myc gene was found in six of eight Burkittlike lymphomas of the intestine. In five of these six cases, a chromosomal translocation t(8;14) with an unusual breakpoint was demonstrated by comigration of the rearranged c-myc and a rearranged JH sequence. This pattern of rearrangement has not been previously associated with a specific group of non-Hodgkin's lymphomas. In contrast to all six low-grade lymphomas, c-myc rearrangements were found in 6 of 12 large-cell or high-grade mucosa-associated lymphomas of the stomach. No comigration of c-myc and immunoglobulin heavy-chain gene sequences were found. We conclude that primary GI lymphomas have different molecular genetic characteristics compared with node-based follicle center-cell lymphoma and as a group are not related to these lymphomas. In addition, the prevalence and patterns of c-myc rearrangements in the gastric large-cell lymphomas and ileocecal Burkittlike lymphomas are noteworthy and suggest a different and distinct pathogenesis for these tumors.

© 1990 by The American Society of Hematology.

PRIMARY EXTRANODAL non-Hodgkin's lymphomas (NHL) differ from nodal NHL with respect to clinical, morphological, and immunologic aspects. Most extranodal lymphomas develop in mucosa-associated lymphoid tissues and especially in the gastrointestinal (GI) tract. While on morphological grounds most gastrointestinal lymphomas (GIL) of the stomach have been regarded as follicle center-cell lymphomas, Myhre and Isaacson showed that the follicular appearance is due to the presence of tumor cells in pre-existent reactive follicles. Recently the concept of mucosa-associated lymphomas (MAL) was introduced as a distinct clinicopathologic entity, and it was suggested that these lymphomas are derived from a separate lymphocyte lineage.

Cytogenetic and molecular analysis has led to the association of specific genetic changes with different types of NHL. Follicular lymphomas are characterized by t(14;18), involving the bcl-2 gene. Most Burkitt's or Burkitt-like lymphomas (BL) are characterized by t(8;14), involving the c-myc gene, and t(11;14), involving the bcl-1 gene, is present in at least 50% of lymphomas of intermediate differentiation (IDL) (reference 8 and M. Raaffeld, unpublished data, January 1990). These translocations presumably reflect differences in ontology of the different types of NHL. In spite of the important differences in behavior and pathology between nodal and extranodal lymphomas, no distinction was made between these lymphomas in genetic or molecular studies published so far.

We have analyzed rearrangements of bcl-1, bcl-2, and c-myc gene by Southern blot analysis in 32 extranodal lymphomas of the GI tract. The differences with nodal lymphomas suggest important differences in tumor ontology, and, in particular, the presence of c-myc rearrangements in 50% of gastric large-cell lymphomas is noteworthy. The findings may be crucial to understanding the differences in the behavior and biology of nodal and extranodal lymphomas.

MATERIAL AND METHODS

Since 1981, all histologically diagnosed NHL in the region covered by the Comprehensive Cancer Centre West in The Netherlands have been registered, including data regarding therapy and survival. All primary GI lymphomas were selected using the criteria of Lewin et al (presentation with GI lymphoma without clinical signs of dissemination).

As described before, all cases were classified according to the Kiel classification, modified to include MAL as an entity, and graded according to the Working Formulation (WF; Table 1). MAL-low grade was defined as a primary mucosal B-cell lymphoma consisting of polymorphic small-to-intermediate large cells, many of which have cleaved nuclei. Using the WF, those cases would be included in the categories of small lymphocytic and diffuse, small cleaved. When groups of larger, centroblast-like cells were present, the lesion was classified as MAL-high and in the WF would be most equivalent to diffuse, mixed, small and large type. Cases solely consisting of centroblasts were designated as centroblastic lymphoma (CB), large-cell lymphomas in the WF. The term "Burkitt-like," used throughout the text, is defined to include all undifferentiated small, noncleaved-cell lymphomas exclusive of classical African Burkitt's lymphoma.

DNA was extracted using standard methods from all cases of which sufficient frozen tissue was available. This series includes 19 patients from the previous study and 13 additional, more recent cases. Using standard methods as described before, Southern blotting was performed after digesting with restriction enzymes EcoRI, HindIII and BamHI. The same filters were hybridized with a 2.4-kb SauIII fragment of JH, a 2.8-kb EcoRI-HindIII fragment of the bcl-2 gene containing the major breakpoint area, and a 3'...
Table 1. Classification of Lymphomas in Study According to Primary Site, the Modified Kiel Classification, and the Grade of the Working Formulation

<table>
<thead>
<tr>
<th>WF</th>
<th>Modified Kiel</th>
<th>Stomach</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>MAL-low</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Inter</td>
<td>MAL-high</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>High</td>
<td>Burkitt-like</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MAL-low, mucosa-associated lymphoma, low grade; MAL-high, mucosa-associated lymphoma, high grade; CB, centroblastic (or large-cell) lymphoma, diffuse.

Table 2. Numbers of Cases Showing Rearrangements for JH, bcl-1, bcl-2 (Major or Minor Breakpoint Region), and c-myc

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>JH</th>
<th>bcl-1</th>
<th>bcl-2</th>
<th>c-myc</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAL-low</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MAL-high</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CB</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BL</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IB</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T-cell</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: MAL-low, mucosa-associated lymphoma, low grade; MAL-high, mucosa-associated lymphoma, high grade; CB, centroblastic (or large-cell) lymphoma, diffuse; BL, Burkittlike lymphoma; IB, immunoblastic lymphoma; T-cell, T-cell lymphoma.

RESULTS

All B-cell lymphomas and one T-cell lymphoma showed at least one rearranged JH fragment, indicating that the sample contained enough tumor cells to be detected by Southern blotting (Table 2, Fig 1). We did not detect a bcl-1 rearrangement in any of our cases. There was no bcl-2 rearrangement found in any of the gastric or intestinal lymphomas, with one exception. This latter case was a large-cell lymphoma of the stomach; there was no comigrating JH fragment, and there was a c-myc rearrangement as well (Fig 1).

Rearrangement of the c-myc gene was detected in 12 cases of B-cell lymphoma of the stomach or intestine. One case of Burkittlike and two cases of large-cell lymphoma were not evaluable for c-myc. Six of eight evaluable BL, all diagnosed in the intestine, had a c-myc rearrangement. In five of these there was comigration with a rearranged JH fragment. Five of ten large-cell lymphomas, all occurring in the stomach, also had a rearrangement of the c-myc gene. None of them showed comigration with a JH fragment. There were no c-myc rearrangements in the low-grade lymphomas, but this gene was rearranged in one of the two MAL with centroblastic progression. Also in this case there was no comigration with a rearranged JH fragment. The two T-cell lymphomas did not show c-myc rearrangement.

DISCUSSION

Malignant lymphomas are traditionally classified using morphological and immunophenotypical criteria. This has led to the recognition of several distinct clinicopathologic entities, of which follicular lymphoma is one of the commonest neoplasms. More than 90% of follicular lymphomas are characterized by a specific chromosomal translocation, t(14;18), which brings the bcl-2 gene, a putative oncogene, under the control of the regulatory genes of the immunoglobulin heavy-chain gene. Large-cell lymphomas form a heterogeneous group that consists partly of tumors that have progressed from low-grade lymphomas, such as follicular lymphoma, as demonstrated by the presence of t(14;18) in about 30% of these lesions. Histologic classifications such as the Kiel-scheme and WF are based primarily on malignant lymphomas arising in lymph nodes. About 40% of the non-Hodgkin's lymphomas arise from extranodal sites, of which the GI tract is the commonest. Traditionally classification of extranodal lymp-
phomas has been based on the assumption that these lesions reflect their nodal counterparts. However, recent clinicopathologic studies have suggested that this assumption may not be correct.\(^1\)\(^2\)\(^3\) In molecular and cytogenetic studies published so far, no distinction between nodal and extranodal lymphomas has been made. Most recently Pan et al\(^6\) showed that t(14;18) is not present in low-grade mucosa-associated lymphoma, although a previously published case with a bcl-2 translocation was not included in the study.\(^7\) In the present study we found important differences between nodal and extranodal lymphomas; in contrast to nodal follicular lymphomas, no bcl-2 rearrangements were found in any of the eight mucosa-associated lymphomas (MAL). Also, in only one of 10 GI large-cell lymphomas was involvement of the bcl-2 gene found. Thus our results indicate that mucosa-associated lymphoma and probably also primary, gastric large-cell lymphoma are not related to follicular lymphoma of the lymph node.

The high frequency of c-myc rearrangements in primary, gastric large-cell lymphomas, which make up more than 50% of all gastric lymphomas,\(^2\) is not found in primary, nodal large-cell lymphoma. No comigration of c-myc with a JH gene was observed in the five gastric large-cell lymphomas. This pattern of c-myc rearrangement most closely resembles those described for sporadic Burkitt lymphomas in which c-myc has rearranged into the switch region of the Ig locus deleting JH.\(^18\) Alternatively, the absence of JH comigration may indicate translocation into a different genetic region other than the Ig locus. Further study to elucidate the nature of these rearrangements is in progress. It might be speculated that in gastric large-cell lymphoma the rearrangement of c-myc is a secondary event related to tumor progression from low-grade MAL, as observed in one case of MAL-high.

The pattern of c-myc rearrangement in ileocolic BL, which shows comigration of the rearranged c-myc fragment with a rearranged JH gene, is distinct from that described for other groups of lymphomas having c-myc rearrangements. Rearrangements of c-myc are almost always associated with a t(8;14) or more rarely a variant t(2;8) or t(8;22). These translocations are characteristic for BL and many AIDS-related lymphomas.\(^18\)\(^17\)\(^19\)\(^20\) Different breakpoints are found in African compared to sporadic cases, suggesting a different origin of the translocation.\(^18\) In African or endemic BLs the breakpoint on chromosome 8 is 'S' from the c-myc gene at a distance that makes detection with a second or third exon probe impossible. The breakpoint on chromosome 14 is generally in the joining region of the immunoglobulin heavy chain.\(^18\) Non-African or sporadic BLs break within the c-myc gene, which can be detected as a rearrangement of the c-myc gene with a second or third exon probe, as used in this study. The chromosome 14 break occurs often within the switch region, and in those cases comigration can be detected.

In summary, our studies of the molecular genetics of GI lymphomas support the growing clinicopathologic evidence that GILs are distinct from nodal lymphomas by showing that there are differences at the molecular level as well. In addition, we have identified what appear to be characteristic molecular associations in two subgroups of GILs: a group of ileocolic lymphomas that have c-myc rearrangements comigrating with JH and a group of gastric large-cell lymphomas that have noncomigration c-myc rearrangements.

REFERENCES

18. Neri A, Barriga F, Knowles D, Macgrath IT, Dalla-Favera...
R: Different regions of the immunoglobulin heavy-chain locus are involved in chromosomal translocations in distinct pathogenetic forms of Burkitt lymphoma. Proc Natl Acad Sci USA 85:2748, 1988


Molecular genetics of gastrointestinal non-Hodgkin's lymphomas: unusual prevalence and pattern of c-myc rearrangements in aggressive lymphomas

JH van Krieken, M Raffeld, S Raghoebier, ES Jaffe, GJ van Ommen and PM Kluin