Microsatellite Instability Is Rare in B-Cell Non-Hodgkin’s Lymphomas


Microsatellite instability (MSI), a symptom of defect in DNA mismatch repair function, represents a type of genomic instability frequently detected in many types of cancers. However, the involvement of MSI in non-Hodgkin’s lymphomas (NHL) has not been conclusively investigated. In this study, we have tested the presence of MSI in 69 cases of B-cell NHL (B-NHL) representative of the various histologic categories of the disease and including 17 cases of acquired immunodeficiency syndrome (AIDS)-related B-NHL (AIDS-NHL). In addition, for selected B-NHL cases, consecutive samples obtained before and after clinical progression (with and without concomitant histologic transformation) were also investigated. Five distinct microsatellite repeats (2 dinucleotide, 2 trinucleotide, and 1 tetranucleotide repeats) were analyzed by polymerase chain reaction in all cases. MSI, defined by the presence of microsatellite alterations in two or more of the five microsatellite loci tested, was not found in NHL. In contrast to a previous study reporting the frequent association between MSI and AIDS-NHL, we found this abnormality in only 1 of 17 cases of AIDS-NHL representative of the major subtypes. Overall, these data indicate that defects in DNA mismatch repair do not contribute significantly to the molecular pathogenesis of B-NHL.

© 1997 by The American Society of Hematology.

MICROSATELLITE INSTABILITY (MSI) represents a specific type of genomic instability associated with human cancers. MSI is defined as the presence of tumor-associated alterations in the germline size of microsatellite repeats and is the result of a reduced efficacy of cellular mismatch repair systems due to mutations in specific DNA repair genes. MSI has been initially identified in approximately 80% of hereditary nonpolyposis colorectal cancers. Subsequently, a large body of evidence has documented that MSI represents one of the most common genetic lesions of human solid tumors, since it occurs at variable frequencies in a wide variety of human sporadic cancers, including those of the colon, lung, stomach, pancreas, endometrium, kidney, bladder, and breast.

The involvement of MSI in lymphoma has not been conclusively investigated. A recent study suggested that, among non-Hodgkin’s lymphomas (NHL), MSI is a peculiar feature of cases associated with acquired immunodeficiency syndrome (AIDS). However, the tumor panel included in this study was numerically limited and was not representative of the various histologic categories and of the different clinical stages of NHL.

To conclusively determine the distribution of MSI throughout the histologic and clinical spectrum of B-cell NHL (B-NHL), we have tested the frequency of this genetic lesion in a panel of well characterized B-NHL at different clinical stages and arising in the immunocompetent host as well as in HIV-infected individuals. Our results indicate that, in contrast to initial suggestions, MSI is not associated with the molecular pathogenesis of B-NHL including AIDS-NHL.

MATERIALS AND METHODS

Tumor biopsies. Fifty-seven primary tumor samples, collected from patients with histologically confirmed diagnosis of B-NHL, were analyzed for MSI. The panel included 40 cases of B-NHL of the immunocompetent host and 17 cases of AIDS-related B-NHL. Among B-NHL of the immunocompetent host, 29 cases were studied at diagnosis, whereas 11 cases were studied after clinical progression (with or without concomitant histologic transformation). In all cases, the amount of malignant cells in the pathologic specimen was at least 70%, as determined by cytologic analysis of cell-surface markers and antigen-receptor gene rearrangement analysis. Genomic DNA of cryopreserved tumor samples (52 cases) was obtained by “salting out” extraction as previously reported. For paraffin embedded specimens (5 cases of AIDS-related B-NHL), DNA was obtained by xylene deparaffinization followed by proteinase K digestion, phenol extraction, and ethanol precipitation.

Tumor cell lines. Twelve cell lines representative of Burkitt’s lymphoma were also analyzed for the presence of MSI. The detailed characterization of these cell lines has been reported previously.

Control DNA samples. Autologous normal DNA from the B-NHL patients included in the panel was obtained from one of these sources: peripheral blood (previously studied by cytofluorimetric analysis of cell surface markers in order to exclude the presence of the malignant clone), primary cultures of fibroblasts obtained from the tumor biopsies, or lymphoblastoid cell lines previously established by Epstein-Barr virus infection of normal B cells present in the B-NHL biopsies. In the case of B-NHL studied after the occurrence of clinical progression (with and without histologic transformation), control DNA was derived from the lymphoma sample obtained before the occurrence of progression.

Analysis of microsatellite instability. Five microsatellite repeat markers mapping to different chromosomes were studied, including 2 dinucleotide repeats (DSS404 and DSS255), 2 trinucleotide repeats (AR and D14S50), and 1 tetranucleotide repeat (FGA) (Table 1). The inclusion of trinucleotide and tetranucleotide repeats is relevant, since MSI in other types of human cancers appears to involve these types of microsatellites with a peculiarly high frequency. Each
microsatellite repeat was amplified by polymerase chain reaction (PCR) and subjected to gel electrophoresis aimed at comparing the pattern of tumor and control DNA from the same patient. Oligonucleotides used as primers were based on sequences derived from the Genome Data Bank (GDB, Baltimore, MD) and were synthesized by Operon (Mountain View, CA). Primers for DSS404 and D8S255 were obtained from Research Genetics (Huntsville, AL).

**PCR amplification and electrophoretic conditions.** PCR reactions were performed in a final volume of 10 µL containing 100 ng of DNA template, 1 to 2 mMol/L MgCl₂, 10 pmol of each primer, after the occurrence of clinical progression. Oligonucleotides used as primers were based on sequences derived from the Genome Data Bank (GDB, Baltimore, MD) and were synthesized by Operon (Mountain View, CA). Primers for DSS404 and D8S255 were obtained from Research Genetics (Huntsville, AL).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Repeat Unit</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS404</td>
<td>Dinucleotide</td>
<td>5</td>
</tr>
<tr>
<td>D8S255</td>
<td>Dinucleotide</td>
<td>6</td>
</tr>
<tr>
<td>D14S50</td>
<td>Trinucleotide</td>
<td>14</td>
</tr>
<tr>
<td>FGA</td>
<td>Trinucleotide</td>
<td>4</td>
</tr>
<tr>
<td>AR</td>
<td>Tetranucleotide</td>
<td>X</td>
</tr>
</tbody>
</table>

**RESULTS**

A total of 69 B-NHL cases was investigated for the presence of MSI. The panel was representative of B-NHL of the immunocompetent host at diagnosis and after clinical progression (11 cases) as well as of AIDS-NHL (17 cases). All samples were tested at five microsatellite loci, including 2 dinucleotide repeats, 2 trinucleotide repeats, and 1 tetranucleotide repeat. Alterations in the size of microsatellites in the tumor sample were revealed as differences in the electrophoretic migration of the tumor DNA as compared with control DNA from autologous tissues. Only tumors showing alterations at ≥2 microsatellite loci were scored positive for MSI. Since single-locus changes in microsatellites are detectable at low frequency (1 to 4 × 10⁻³ per cell generation) in nontumor DNA in the absence of detectable defects in DNA mismatch repair, this threshold would significantly lower the probability of detecting background mutations.

**B-NHL at diagnosis.** Forty-one cases of B-NHL of the immunocompetent host (29 biologic specimens and 12 tumor cell lines) were investigated at diagnosis. The panel was representative of the major B-NHL histologic categories and included small lymphocytic lymphoma, follicular lymphoma, diffuse large cell lymphoma, and Burkitt’s lymphoma. The results are summarized in Table 2 and representative cases are illustrated in Fig 1A. Although five cases showed alterations at only one microsatellite locus, no case exhibited alterations at ≥2 loci among the five microsatellites tested. Thus, MSI was scored negative in all 41 B-NHL of the immunocompetent host studied at diagnosis.

**B-NHL after clinical progression.** For 11 cases of B-NHL of the immunocompetent host, the presence of MSI was investigated in pairs of consecutive samples obtained before and after clinical progression. Clinical progression was defined as enlargement of adenopathy, involvement of new sites, or stage progression. For these same 11 patients, the clonal identity of the lymphoma samples obtained before and after clinical progression has been defined in a previous study. In three cases, clinical progression was accompanied by morphologic evidence of transformation from a follicular to a diffuse histology. The results of MSI analysis of clinically progressed B-NHL are summarized in Table 3 and representative results are shown in Fig 1B. Two patients showed alterations at one single microsatellite locus in the lymphoma sample obtained after clinical progression, whereas in all other samples the microsatellite pattern detected before and after progression was identical. Accordingly, MSI was scored negative in all 11 B-NHL studied after the occurrence of clinical progression.

**AIDS-related B-NHL.** Seventeen cases of AIDS-related B-NHL were studied, including 9 large noncleaved cell lymphomas, 2 large cell immunoblastic-plasmacytoid lymphomas, and 6 Burkitt’s lymphomas. One case (TNC69), represented by an AIDS-related Burkitt’s lymphoma, showed microsatellite alterations at three of five tested loci, thus fulfilling the criteria for MSI. In all remaining cases of AIDS-related B-NHL, no microsatellite alterations were detected (Table 4).

**DISCUSSION**

This study demonstrates that the molecular pathogenesis of B-NHL does not involve MSI. The absence of MSI in B-NHL is independent of the host’s immune function, since both B-NHL of the immunocompetent host and AIDS-related B-NHL are devoid of MSI in virtually all cases. In addition, when considering the marked degree of heterogeneity of B-NHL, it is notable that lack of MSI involvement is a consistent feature throughout the entire B-NHL clinicopathologic spectrum, which includes indolent lymphomas characterized by a low proliferative index and a slow clinical course, such as small lymphocytic lymphoma and follicular lymphoma, as well as high-grade lymphomas characterized by a high proliferative index and an aggressive clinical course, such as diffuse large cell lymphoma and Burkitt’s lymphoma. Interestingly, it has been recently reported that

**Table 2. Frequency of Microsatellite Alterations in B-NHL at Diagnosis**

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. of Cases Tested</th>
<th>Cases Showing Microsatellite Alterations*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 1 Locus</td>
<td>At ≥2 Loci</td>
</tr>
<tr>
<td>Small lymphocytic lymphoma</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Diffuse large cell lymphoma</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Burkitt’s lymphoma</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

* Five microsatellite loci were tested in each case.
MALT lymphoma (not included in this study) display MSI at high frequency using criteria for defining MSI analogous to this study, suggesting that these tumors may be associated with distinct pathogenetic mechanisms. Finally, our data show that not only MSI is not involved in B-NHL at diagnosis, but it is also not responsible for the clinical progression and histologic transformation that may occur in the late phases of the natural history of these tumors.

The lack of association between MSI and B-NHL development, as indicated by our survey, is consistent with other findings obtained in experimental mice and in related lymphoid malignancies. Mice carrying homozygous deletions of the \( MSH2 \) gene, whose wild type protein product protects the cellular genome from the occurrence of DNA replication errors including microsatellite alterations, do not develop B lymphomas, but rather T-cell lymphomas characterized by MSI. The observation that the \( MSH2^{0/0} \) genotype does not lead to B-NHL development confirms the notion that these lymphoid malignancies are not associated

---

**Table 3. Frequency of Microsatellite Alterations in B-NHL of the Immunocompetent Host After Clinical Progression**

<table>
<thead>
<tr>
<th>Type of Lymphoma</th>
<th>No. of Cases Tested</th>
<th>Cases Showing Microsatellite Alterations*</th>
<th>At 1 Locus</th>
<th>At ( \geq 2 ) Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>With histologic transformation†</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Without histologic transformation</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Clinical progression was defined by enlargement of adenopathy, involvement of new sites or stage progression (Lo Coco et al, 1993).²⁶

* Five microsatellite loci were tested in each case.

† Histologic transformation was defined as B-NHL evolution from follicular to diffuse large cell architecture.

**Table 4. Frequency of Microsatellite Alterations in AIDS-Related B-NHL**

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. of Cases Tested</th>
<th>Cases Showing Microsatellite Alterations*</th>
<th>At 1 Locus</th>
<th>At ( \geq 2 ) Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large noncleaved cell lymphoma</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Large cell immunoblastic-plasmacytoid lymphoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Burkitt’s lymphoma</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Five microsatellite loci were tested in each case.
with defects in DNA mismatch repair systems. The second set of data is constituted by the extreme rarity of MSI in B-cell chronic lymphocytic leukemia (B-CLL), a B-cell malignancy sharing several features with some histologic categories of B-NHL. Indeed, although MSI has been occasionally reported in association with B-CLL, its frequency in this disease is less than 5%, as derived from cumulative data of published B-CLL series.

With respect to AIDS-NHL, the results presented in this study are apparently in contrast suggesting a high rate (4 of 6) of MSI in a panel of AIDS-NHL. Although the histology of the cases analyzed is not reported in that study, it is unlikely that the discrepancy in the results is due to the overrepresentation of any given type of AIDS-related NHL associating with MSI since our analysis indicates the absence or rarity of MSI throughout all major histologic categories of AIDS-NHL. Conversely, the discrepancy may have derived from the different criteria used by the two studies to score MSI since Bedi et al scored as positive for MSI also cases showing abnormalities at only one microsatellite locus, whereas we adopted a more restrictive definition of MSI positivity. Finally, the number of cases tested in the previous study is very small and may have led to an overestimation of an otherwise rare phenomenon.

Overall, the absence of MSI in B-NHL, as opposed to the high frequency of this genetic lesion in solid cancers, particularly those of epithelial origin, represent one additional example of the distinct genetic mechanism underlying the pathogenesis of lymphoid malignancies. Solid tumors of epithelial origin tend to show random chromosomal alterations that can affect more than 20% of the entire genome, as well as defects in DNA mismatch repair genes, which may produce more subtle alterations throughout the genome. Conversely, lymphoid malignancies have been traditionally recognized as tumors characterized by a relatively stable genome and by a restricted panel of well defined recurrent chromosomal abnormalities, among which chromosomal translocations are particularly frequent. The molecular mechanism underlying these lesions remains unknown.

REFERENCES

Microsatellite Instability Is Rare in B-Cell Non-Hodgkin's Lymphomas