A Clonally Distinct Recurrence of Burkitt’s Lymphoma at 15 Years

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A human immunodeficiency virus-negative male was successfully treated for two occurrences of Burkitt’s lymphoma, 15 years apart. As consolidation of his second remission, he underwent high-dose chemotherapy with peripheral blood stem cell transplantation. In an effort to prove whether the second lymphoma was a relapse of the first or a second primary lymphoma, we obtained paraffin-embedded material from both lymphomas. DNA was extracted from this material and amplified by polymerase chain reaction (PCR) using consensus JH and Vμ region primers. Analysis of the PCR products, which mostly reflect VDJ joints, showed two sharp bands of different molecular size, proving the monoclonal nature of the lymphomas and suggesting that each had different Ig gene rearrangements. Sequencing of both PCR products showed a marked dissimilarity in nucleotide sequence in the clonally unique VDJ joint region, providing strong evidence for the separate cellular genesis of each lymphoma. These results suggest that late relapses of Burkitt’s lymphoma should be examined for clonal distinctiveness. If the second lymphoma is distinct from the primary one, it might be treated as a primary lymphoma rather than as recurrent disease.

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Fig 1. Photomicrograph of BL from 1978 and 1993. Histological sections of the retroperitoneal masses from 1978 (A) and 1993 (B) showed Burkitt-like small noncleaved follicular center cell lymphoma. Note the similarity in histological appearance between the two specimens. The lymphoma from 1993 did show a greater number of apoptotic cells overall.

Tris-HCl, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 400 μg/mL Proteinase K, 1% sodium dodecyl sulfate) overnight at 55°C. After 10 minutes of incubation at 95°C and centrifugation at 4°C, 4 μL of digest was amplified for 37 cycles using 5' consensus Ig heavy-chain variable (V<sub>H</sub>) and 3' joining (J<sub>H</sub>) region primers in a total volume of 50 μL, using standard conditions as described. The primers, which are shown in Fig 3, are designed to specifically amplify most Ig heavy-chain VDJ joints or CDR3 regions that serve as clonal markers because of their marked variability. After amplification, approximately one third of the resultant PCR products was electrophoresed in 8% polyacrylamide gels and was visualized by staining with ethidium bromide.

DNA sequencing. PCR products were purified using Wizard PCR Preps (Promega, Madison, WI) and cloned using the pCR-Script Cloning Kit (Stratagene, La Jolla, CA) following the manufacturer’s directions. Plasmid DNA was isolated from overnight cultures of randomly selected white colonies (those that contained plasmids with inserts) using Wizard Minipreps (Promega). Dideoxynucleotide sequencing was performed with Sequenase (US Biochemical, Cleveland, OH) following the manufacturer’s protocol for a double-stranded plasmid, using approximately one third of the isolated DNA and the M13 reverse primer.

RESULTS

Histopathology

The histological sections from 1978 showed an ulcerated portion of bowel with a transmural lymphoid infiltrate most consistent with a Burkitt-like small noncleaved follicular center cell lymphoma (Fig 1). Numerous mitotic figures, apoptotic cells, and tingible body macrophages created a starry-sky appearance. Paraffin-section immunoperoxidase stains again confirmed the B-cell origin of the neoplastic cells (CD20<sup>+</sup>, CD3<sup>−</sup>). A staging bone marrow aspirate and biopsy specimen did not show lymphoma.

Molecular Genetic Characterization

DNA extracted from both tumors was amplified with the consensus V<sub>H</sub> and J<sub>H</sub> primers, and the resultant products were...
electrophoresed under conditions that permitted resolution of small differences in size. As shown in Fig 2, an intense, single band, indicative of a dominant monoclonal population was observed for both the 1978 and 1993 specimens. As expected, the polyclonal control specimen produced a broad ladder of faint bands reflecting the different size VDJ joints. The different sizes of these bands suggested that the two lymphomas were different clones. To confirm these results, the PCR products from both lymphomas were cloned, and three randomly selected clones from each were sequenced. With the exception of one point mutation in a single clone, the three clones analyzed from each specimen had identical VDJ joint sequences, indicating that they represent clonal B-cell expansions. Moreover, as shown in Fig 3, the VDJ joint sequences of the two lymphomas are different, but both are potentially functional genes because they are in-frame and do not generate stop codons. Because there are over 30 diversity gene segments (D), unrelated clones would probably use different D segments, which appears to be the case here. None of the known D segments has significant homology with the 1978 D region. However, the 1993 D region matches in eight of nine positions with the Dlr2 segment. Both lymphoma heavy chains appear to be using J4, which is the most commonly used of the six potentially functional JH segments, being found in more than 50% of heavy-chain rearrangements. Thus, usage of the same J4 segment does not provide evidence for identity between two clones.

**DISCUSSION**

Most relapses of BL occur within the first year from complete remission. Identity of Ig gene rearrangements between initial and recurrent disease would prove that both originated from the initial lymphoma. Sufficient dissimilarity would strongly suggest two separate clonal origins of initial and recurrent disease. Although somatic mutation may result in alterations in the VDJ region, in our case, the sequence differences identified are sufficiently great to suggest that this is only a remote possibility. Moreover, there is little evidence for somatic mutation of VH genes occurring in vivo within any BL. Although Chapman et al found occasional sequence changes in one of five BL cell lines examined, the CDR3 sequence remained essentially unchanged. In contrast to follicular lymphoma, it follows that there is little evidence for clonal evolution in BL. Thus, the VH gene nucleotide sequence is relatively fixed and provides a reliable clonal signature for BL.

Using IgM, κ and glucose-6-phosphate-dehydrogenase isoenzyme phenotyping, in an era before DNA typing methods were available, Fialkow et al compared initial and recurrent BLs from 26 patients. In 2 of 31 instances, the phenotypes were discordant for IgM and κ-light chain expression, and in 1 of 8 instances were discordant for glucose-6-phosphate-dehydrogenase phenotype. All cases of concordance occurred with relapse before 5 months of remission duration, and all cases of discordance occurred after 5 months of remission. They hypothesized that discordant cases might be because of the emergence of a second malignant clone.

Barriga et al reported an HIV-positive patient whose BL recurred 27 months after achieving complete remission. Restriction enzyme analysis of the c-myc and Ig heavy-chain loci in the original and recurrent tumor showed two distinct clones. Different breakpoints within c-myc and different mutations in the regulatory or coding regions between initial and recurrent tumors would provide further evidence for their clonal distinctiveness. Alternatively, identical breakpoints would suggest that the two clones are still related and share early genetic events that contribute to the malignant phenotype. Although we did not show translocation of c-myc, the histopathology clearly shows BL. Mutations of p53 have been reported in some BL. Sequence differences or discordant p53 protein expression would add to the evidence that the initial and recurrent BLs had distinct clonal origins.

The patient reported here had no known predisposing factors for the development of lymphoma yet has developed two clonally distinct BLs. Specifically, there is no history of lymphoproliferative disease, nasopharyngeal carcinoma, chronic myelogenous leukemia, or other cancer in his family, nor has there been an increase in severity of infection with Epstein-Barr virus (EBV). His socioeconomic level is on the lower end of that of American society, yet he maintains adequate housing, sanitation, nutrition, and health care. It is unknown if he had been exposed to EBV before the acquisition of his first lymphoma and, as was expected, had positive serology against EBV at his second diagnosis. However, as would be expected for most nonendemic BL, both tumors were negative for EBV as assessed by EBV-encoded RNA-in-situ hybridization (EBER-ISH; data not shown).

Observations presented in this report are important, because they confirm that this very late recurrence is caused by the development of another malignant clone rather than a recrudescence of the originally treated lymphoma. Moreover, this is the first report from VDJ sequence evidence, that two BLs developing in the same patient are clonally distinct. In addition, the 15-year interval between the two lymphomas is also the longest period of complete remission followed by relapse thus far described.

Although uncertain, identification of a clonally distinct
recurrence of BL suggests that it might be treated as a primary occurrence of tumor. This is an important distinction with regards to the prescription of treatment in such individuals and also for the analysis of experimental therapy. Because the incidence of clonally distinct relapse is unknown, at a minimum, late recurrences of BL should be examined for clonal identity with the initial tumor.

REFERENCES

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