The bcl-2 Gene, Follicular Lymphoma, and Hodgkin’s Disease

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In this issue of the Journal, Stetler-Stevenson et al. (1) describe studies that have potentially profound implications for our understanding of the biology of Hodgkin’s disease, an entity with long-time enigmatic pathogenesis and cellular origin. Stetler-Stevenson and her colleagues show that within the tissues of at least one third of unselected Hodgkin’s disease patients are cells carrying a specific DNA abnormality that is the molecular hallmark of the 14;18 chromosomal translocation. This translocation, which results in deregulation of the so-called bcl-2 gene on chromosome 18, had previously been restricted to bona fide B-lineage neoplasms, particularly non-Hodgkin’s follicular lymphomas. The authors (1) deduce that their results imply a potential B-cell origin for the characteristic Reed-Sternberg cell and implicate the bcl-2 gene product in the pathogenesis of a clinicopathologic entity distinct from low-grade non-Hodgkin’s lymphomas. These results, to say the least, are certainly unexpected and most provocative.

The technique used by Stetler-Stevenson and associates was the polymerase chain reaction (PCR) that recently has seen an explosive increase in its application to a wide variety of investigative problems in molecular biology and medicine. In this technique, repetitive cycles of specifically primed DNA synthesis are used to amplify a target sequence up to one billion-fold. However, the inherent power of the technique to detect minute amounts of the target DNA molecule is offset by the specter of false-positive signals resulting from subpicogram amounts of contaminating DNA. These minute amounts of contaminating DNA are widespread and present serious problems to laboratory researchers using the PCR technique to examine materials that may have low levels of the target sequence; they must take special precautions and perform the technique meticulously to minimize possible cross-contamination of test samples. Contamination does not appear to account for the findings of Stetler-Stevenson et al., because they were able to repeat their results with freshly extracted tissues in an independent laboratory. In addition, they performed several control measures to rule out possible contamination of reagents. Therefore, the experimental methods used appear sound, and the presence of cells carrying the 14;18 chromosomal translocation in tissues of patients with Hodgkin’s disease must be addressed.

A potential explanation for the current observations is that the examined tissues contained both Hodgkin’s disease and follicular lymphoma. Indeed, the authors acknowledge that several of their “positive” tissues were found on review to contain composite lymphomas consisting of Hodgkin’s disease and non-Hodgkin’s follicular lymphomas. Can their results be explained by the possibility that 30%-50% of unselected Hodgkin’s disease cases represent occult composite lymphomas? The previously reported incidence of this phenomenon that was based on histologic examination of tissues from staging laparotomies was no higher than 5%. Furthermore, long-term disease-free survival of patients with Hodgkin’s disease is inconsistent with a high incidence of occult composite lymphomas, because the follicular component would likely not be cured by standard therapies for Hodgkin’s disease. However, it is conceivable that a follicular low-grade lymphoma, so occult as to be identifiable only by the PCR technique, may not have the same natural history or potential as the disease when it is morphologically or clinically apparent.

The intriguing coexistence of these two entities at higher than expected frequencies may be compatible with a shared pathogenesis, as suggested by the authors. If so, then secondary events in addition to bcl-2 translocation may account for the different clinicopathologic manifestations of these two types of lymphoma. The bcl-2 does not appear to be a particularly potent oncogene; it has subtle effects on the growth properties of B lymphocytes, as demonstrated by numerous gene transfer experiments. These properties, however, are compatible with the indolent character of 14;18-associated B-cell lymphomas and the importance of secondary cytogenetic changes in determining their clinical behavior.

An issue that is unsettled concerns which cells in Hodgkin’s disease tissues contain the t(14;18). At this point, no data convincingly demonstrate that it is the Reed-Sternberg cells. Stetler-Stevenson and her associates did not correlate PCR signal intensity with proportion of estimated Reed-Sternberg cells and variants; however, the data they presented suggest that one of 1,000 examined cells carried the 14;18 fusion, which approximates the expected abundance of these cells. It should be possible for one to obtain preparations enriched for Reed-Sternberg cells and test for a corresponding increase in the PCR signal intensity. A hundred-fold enrichment should also allow detection of bcl-2 gene rearrangements by Southern blot analysis that would provide valuable confirmation of the PCR results. Such preparations of Reed-Sternberg cells have, in the past, been shown to contain cells with clonal immunoglobulin gene rearrangements, and comigration of these with bcl-2 rearrangements would be pathognomonic for the 14;18. Alternatively, in situ studies in which bcl-2 DNA or antibody probes are used may show that the Reed-Sternberg cells contain abundant bcl-2 RNA or protein that we know to be highly expressed in neoplastic B cells with a t(14;18). If we are to appreciate more fully the significance of the current report, it is imperative that investigators demonstrate that Reed-Sternberg cells carry the 14;18.

If it is not the Reed-Sternberg cells that carry the molecular abnormality detected by Stetler-Stevenson et al., perhaps it is an occult B-cell population that is not overtly malignant but yet carries the 14;18 translocation. These incompletely transformed cells may be more prevalent in patients with Hodgkin’s disease...

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who are relatively immunosuppressed and perhaps deficient in various immune surveillance mechanisms. By analogy, B-cell proliferations containing translocated myc genes arise at high frequency in immunosuppressed organ allograft recipients. Perhaps the differences in degree and nature of immunosuppression allow for clonal outgrowth (overt or occult) of cells with translocations that require additional events for complete development of tumorigenicity as suggested above.

Cytogenetic studies of Hodgkin's disease are not inconsistent with the findings of Stetler-Stevenson and co-workers. Several reports have shown that a small fraction of fresh tumor samples from patients with the disease contains cells carrying a 14q+ marker chromosome, frequently with breakpoints in band 14q32. Furthermore, some cell lines that may be representative of Reed-Sternberg cells have also shown 14q+ marker chromosomes. Only one example of a t(14;18) in Hodgkin's disease has been documented, but the reported low frequency may reflect the difficulty in performing karyotype analyses on tissues in which the neoplastic cell being analyzed represents 0.1%-1% of the total cells. Thus the high frequency of 14;18 in Hodgkin's disease reported by Stetler-Stevenson et al. is in marked contrast to that reported cytogenetically and will need to be reconciled in future studies.

The results of Stetler-Stevenson and associates, though tantalizing, raise several additional questions. Like many of the studies on Hodgkin's disease over the years, the current results seem plausible but yet inconsistent with previous notions of the pathobiology of this lymphoma. As presently identified, it is probably not a single entity by morphologic, clinical, or epidemiologic characteristics. These studies should be analyzed or repeated for more homogeneous types of disease. In the absence of confirmatory data obtained by independent techniques, it is difficult for one to judge the importance and validity of the current observations. If confirmed by analyses of additional tissues by researchers in other laboratories, these observations will stand as a truly significant advance in our understanding of Hodgkin's disease.

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