Patterns of Chromosomal Breakpoint Locations in Burkitt's Lymphoma: Relevance to Geography and Epstein-Barr Virus Association

By Bruce Shiramizu, Francisco Barriga, Janet Neequaye, Ayesha Jafri, Riccardo Dalla-Favera, Antonino Neri, Marina Gutierrez, Paul Levine, and Ian Magrath

We have examined, by Southern blotting, the patterns of chromosomal breakpoint locations in 55 cases of Burkitt's lymphoma (BL) with respect to geography and Epstein-Barr virus (EBV) association. We have confirmed the association between chromosome 8 breakpoint and geography: 74% of endemic (eBL) but only 9% of sporadic BL (sBL) had breakpoints outside the HindIII fragment encompassing the c-myc gene (P < .00001). Conversely, not only did 91% of sBL manifest a rearranged HindIII fragment, but at least 56% of these cases, in contrast to 17% of eBL cases, had a breakpoint within the first exon or intron of c-myc (P < .004). Breakpoints outside the switch μ (Spμ) region (ie, the HindIII fragment encompassing Spμ) on chromosome 14 were twice as common overall (73%) as those within Spμ (27%), but in the 15 tumors with Spμ breakpoints, 13 (87%) had a rearranged c-myc gene. Breakpoints outside the HindIII fragment encompassing c-myc on chromosome 8 were predominantly associated with non-Sp breakpoints on chromosome 14 (85%) and this was the combination most frequently associated with eBL (65%; 6% of sBL, P < .00001). In sBL, the most frequent breakpoint combination was a rearranged c-myc gene with a non-Spμ breakpoint (53%; 13% of eBL). Twenty-eight percent of sBL and 13% of eBL had breakpoints both within c-myc and within Spμ. EBV DNA was present in 19 of 20 tumors with breakpoints outside c-myc, in none of 7 with a breakpoint in the immediate 5′ region of c-myc, in 4 of 5 tumors with breakpoints in the first exon, and in 7 of 12 tumors with breakpoints in the first intron. These data suggest that the pathogeneses of eBL and sBL differ with regard to the mechanism of c-myc deregulation, and probably also with regard to the state of differentiation of the target cell for malignant transformation. We have formulated a testable hypothesis regarding the potential role of EBV in pathogenesis: that it is required to contribute to the deregulation of c-myc in the presence of some, but not all, types of c-myc damage arising from the chromosomal translocations.

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Burkitt's Lymphoma (BL) is a histological subtype of small, noncleaved lymphoma that occurs predominantly in the first two decades of life. The tumor cells invariably contain reciprocal chromosomal translocations, the most frequent of which (occurring in some 80% of cases) is an 8q24;14q32 translocation. The remainder (so-called variant translocations) are either 8q24;22q11 or 2q12;8q24 translocations. In all three types of translocation the breakpoint on chromosome 8 differs in BL in Equatorial Africa (usually referred to as the endemic variety [eBL], reflecting its relatively high incidence in this geographic region) compared with BL in the United States (usually referred to, along with European cases, as the sporadic variety [sBL], reflecting its relatively low incidence in these regions). These data suggested that there are several different mechanisms whereby the c-myc oncogene is deregulated. Differences in the breakpoint patterns may also reflect, and perhaps are a consequence of, differences in the state of differentiation of the cell in which the translocation occurred.

The breakpoint location on chromosome 8 and, consequently, the physical relationship of the translocated c-myc gene to regulatory elements located in its 5′ flanking sequences, as well as to the enhancer elements of the Ig heavy-chain locus (including other potential regulatory elements such as the Ig switch regions), can be determined to within small regions of the relevant genes by Southern blot analysis. With this objective, we have undertaken a comprehensive analysis of 55 BL cell lines and tumors—considerably more than we have previously reported—and have determined the breakpoint regions on both chromosomes 8 and 14, as well as the EBV association for each sample. We have confirmed the relationship of the breakpoint location on chromosome 8 to geography, but were unable to demonstrate a definite relationship between the breakpoint location on chromosome 14 and geography. However, we have extended previous observations by demonstrating an association between the breakpoint locations on chromosomes 8 and 14, and by showing that combinations of breakpoint locations were predominantly associ-
ated with either eBL or sBL. Some breakpoint locations appeared to be predominantly associated with Epstein-Barr virus (EBV)-positive tumors, whereas others were invariably present in EBV-negative tumors, suggesting not only that EBV may have a direct pathogenetic role, but that it may participate in the deregulation of c-myc.

MATERIALS AND METHODS

Samples and Cell Lines

Representative tumor biopsy and/or involved bone marrow specimens were collected from untreated patients. Fresh BL samples originated from Ghana and the United States through the National Cancer Institute’s Burkitt Tumor Project in Ghana and the Pediatric Branch, NCI, or the American BL tumor registry, respectively. Approval was obtained from the Institutional review board for these studies. Patients were informed that part of blood and/or bone marrow or tissue samples would be used for research purposes, and that their privacy would be protected. A small number of the American tumors were diagnosed histologically as small, noncleaved cell lymphoma, and not specified as BL. For the purposes of this analysis, however, all were considered to be BL. All samples were demonstrated to be of B-cell origin by virtue of Ig J heavy-chain rearrangement. Cell lines were largely derived in this laboratory and have been described previously. Cell lines with variant translocations were not included in the analysis.

DNA Extraction

DNA was prepared by cell lysis, digested with proteinase K, extracted with phenol/chloroform, and precipitated with ethanol according to standard techniques.

DNA Probes

The c-myc probes and immunoglobulin probes used in the analysis are shown in Fig 1 and described elsewhere. The presence of EBV DNA was determined by hybridizing the Southern blots with a probe derived from the BamHI K fragment of the EBV genome. EBV-positive tumors were also examined with probes from the terminal repeat region of EBV, which confirmed both positivity and clonality in all cases. Purified DNA fragments were 32P labeled by nick translation or random primer extension.

Southern Analysis

Southern blotting was performed as previously described. The strategy used to determine breakpoint locations entailed the demonstration of rearrangements of c-myc and λ using multiple restriction enzymes and probes. We also sequentially hybridized the same blots with different probes to detect comigration (ie, presumptive identity of) rearranged fragments hybridized with probes derived from chromosomes 8 and 14. The assignment of breakpoint locations was always based on several separate restriction digests hybridized with different probes (Fig 1). Assignments were made when the following conditions were met.

Chromosome 8 Breakpoints

Breakpoint outside c-myc. Tumors or cell lines were considered to have breakpoints outside the c-myc gene (ie, upstream of the S' HindIII site) (Fig 1), when there was germline configuration of the c-myc gene demonstrated by HindIII digests hybridized with both first and third exon probes. In such tumors, Pst I and Pvu II fragments detected with a first exon probe were also in germline configuration, except in those cases in which there was a mutation in or near the Pvu II site in the first exon, as has been previously reported. This mutation results in the production of a rearranged

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**Fig 1.** Restriction map of the human c-myc (chromosome 8) and Ig heavy chain (chromosome 14) loci that are involved in the 8:14 translocations in BL. Restriction endonuclease sites as well as the fragments used as primary first and third exon probes are shown. An Smal–Pvu II fragment was also used as a first exon probe in Smal digests. H, HindIII; Ps, Pst I; C, Cla I; Pvu, Pvu II; S, Sma I; N, Not I; X, Xho I; Ba, Bspmi II; R, Rsr II; B, Bam I; Sa, Sac I; Xb, Xba I; Nh, Nhe I; E, EcoRI; Ba, BamHI.
Pvu II fragment of 1.7 kb instead of the 1-kb germline fragment because the DNA is not cut at the exon site and the fragment extends to the next downstream Pvu II site (Fig 2A).

It should be pointed out that tumors with a breakpoint 3' of the 3' HindIII site (i.e., those with variant chromosomal translocations) would also be included in this category unless rearrangement of the heavy-chain locus were demonstrated.

Rearrangements within the HindIII fragment encompassing c-myc were further localized by determining whether the Pst I and Pvu II fragments in the 5' region of the gene were intact.

Fig 2. Representative Southern blots showing rearranged and germline bands and the associated maps of the rearranged c-myc gene (see Materials and Methods for interpretation of the Southern blots). The germline band in each case is shown in the control lane. Sizes of germline bands are as follows: HindIII, 11.6 kb; EcoRI, 12.8 kb; Pst I, 3.0 kb; Pvu II, 1.0 kb; Smal, 1.8 kb. (A) Breakpoint location outside the HindIII fragment that contains c-myc. No rearrangements are observed with any of the probes or restriction enzymes used. (B) Breakpoint located between HindIII and Pst I. The rearranged band seen with HindIII is identical in size with first and third exon probes, and Pst I shows no rearrangement. (C) Breakpoint located between Pst I and Pvu II. The gene is clearly rearranged with exons I and III both present in the same HindIII fragment, but Pst I shows a rearrangement, whereas Pvu II does not.
BREAKPOINT LOCATIONS IN BURKITT'S LYMPHOMA

Fig 2. (Cont'd). (D) Breakpoint located between Pvu II and Sma I or Sma I and Pvu II. Both tumors (2218 and RAMOS) show rearrangements with HindIII and Pst I, but Sma I digestion shows a rearrangement (and hence a breakpoint in the exon) only in tumor 2218. (E) Breakpoint located between Pvu II and Pst I (intron). HindIII digests probed with first and third exon probes show rearranged bands of different sizes, consistent with separation of exons 1 and 3 in the genome. This is confirmed by the presence of a rearrangement with Pst I, but not with Pvu II.

HindIII-Pst I breakpoint. Tumors in which the rearranged fragments detected in HindIII digests probed with first and third c-myc exon probes were the same size (ie, both exons were in the same HindIII fragment) were considered to have breakpoints between the 5' HindIII site and the first exon. If both Pst I and Pvu II fragments were intact, the breakpoint was considered to be between the 5' HindIII site and the Pst I site immediately 5' of the first exon (Fig 2B).

Pst I–Pvu II breakpoint. A rearrangement of the Pst I fragment but not the Pvu II fragment localized the breakpoint to the small region between Pst I and Pvu II in the 5' flanking sequence of c-myc (Fig 2C).

Pvu II–Sma I breakpoint and Sma I–Pvu II breakpoints. Rearrangement of both Pst I and Pvu II fragments indicated a breakpoint within the 5' Pvu II fragment that includes 5' flanking sequences and the bulk of the first exon. Such breakpoints were further designated as within the exon (Sma I–Pvu II) if rearrangement was detected in an Sma I digest probed with a first exon probe (the Sma I–Pvu II fragment), and immediately 5' (Pvu II–Sma I) if the Sma I fragment was in germline configuration (Fig 2D).

Pvu II–Pst I. A breakpoint within the first intron was documented by the demonstration of different sized HindIII fragments probed with first and third exon probes (ie, the fragments did not comigrate, indicating transection of the gene by the translocation such that first and third exons have come to reside on different chromosomes). This finding was always confirmed by the demonstration of a rearrangement in Pst I but not Pvu II digests when hybridized with a first exon probe (Fig 2E).

Chromosome 14 Breakpoints

Switch μ. The locations of the breakpoints on chromosome 14 were determined by similar analyses using probes from the Ig locus. Breakpoints were classified as within the switch μ (Sμ) region (although they could have been immediately 5' or 3' of the Sμ region proper) in the presence of a lack of comigration of one of the rearranged bands detected with Jμ and Cμ probes in BamHI digests, demonstrating that these regions were not contiguous on one of the chromosome 14 alleles (ie, that there was a translocation breakpoint between them). In addition, for designation as an Sμ breakpoint, a rearrangement of the HindIII fragment encompassing Sμ and Cμ was required. The finding of a rearranged HindIII fragment alone was not considered to be sufficient evidence of an Sμ breakpoint, because small deletions involving the 5' HindIII site itself (therefore resulting in a rearranged band in HindIII digests using Sμ or Cμ probes) have been described in normal cells with rearranged immunoglobulin genes. On occasion, tumors with Sμ breakpoints also showed two rearranged bands when EcoRI or...
The detection of an Sp breakpoint excludes the possibility that the chromosomal translocation is of the variant type. Non-switch μ. C10.5. Comigration of all rearranged bands detected with Jβ and Cμ in BamHI digests, particularly when associated with a germline HindIII fragment probed with Cμ and Sp demonstrated that the chromosome 14 breakpoint was highly unlikely to be within the Sp region. Such breakpoints could have been either upstream of the Sp region (ie, in V, D, or Jβ), downstream of Cμ (ie, within another heavy-chain switch region) or on chromosomes 22 or 2. In some tumors, the presence of two rearranged bands when HindIII, EcoRI, or BamHI digests were hybridized with a Jβ probe confirmed that the Jβ region itself was transected, and the two resultant fragments were on separate chromosomes. Detection of a Jβ breakpoint also excludes a variant translocation.

**Statistical Analysis**

All statistical comparisons were made using the χ² test with two-sided P values.

**RESULTS**

The breakpoint locations of each tumor or cell line examined, EBV association, and karyotype are shown in Table 1.

### Chromosome 8

There was a clear correlation with geographic origin of the tumor: 74% (17 of 23) of cEBL had breakpoints outside the HindIII fragment encompassing c-myc, whereas 91% (29 of 32) of sBL had breakpoints within or close to c-myc (ie, within the HindIII fragment) (Table 2). This difference was highly significant (P < .00001). In all of the tumors with a breakpoint outside the HindIII fragment except one (EB3), the EcoRI fragment encompassing c-myc was also unrearranged, indicating that the breakpoint was also outside the EcoRI fragment that encompasses c-myc. In the case of EB3, the breakpoint was considered to be between the 5′ EcoRI and HindIII sites. Further subdivision of sBL according to locations within the 5′ region of the HindIII fragment (Fig 3) showed that 6% (2 of 32) were between HindIII and Pst I, 13% (4 of 32) between Pst I and Pvu II, 34% (11 of 32) in the Pvu I fragment, and 37% (12 of 32) between Pvu II and Pst I (first intron). Thus, 27 of 32 sBLs (84%) had breakpoints within the Pst I fragment encompassing the 5′ region of the gene. Two of the 23 cEBLs (9%) had breakpoints within the HindIII–Pst I fragment, 3 (13%) within the Pvu II–Pst II fragment, and 1 (4%) had a breakpoint within the first intron, a total of 6 (26%) within the Pst I fragment.

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*AF indicates an endemic tumor obtained from Ghana, AM a sporadic tumor from the United States.

†Samples obtained from a tumor biopsy (T) or a cell line (C).

‡Breakpoint location on chromosome 8. A star indicates the presence of a mutation in the Pvu I site in the first exon. Breakpoints are indicated as being within a DNA fragment bounded by the enzyme sites shown.

§Breakpoint location on chromosome 14. Breakpoints are listed as being within a DNA fragment bounded by the enzyme sites shown.

ID indicates the breakpoint has been deduced by demonstration of a breakpoint location on chromosome 14.
Table 2. Breakpoint Locations on Chromosomes 8 and 14 and EBV Association in Endemic and Sporadic Burkitt’s Lymphomas

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<td>2 (5%)</td>
<td>29 (91%)</td>
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<td>10 (21%)</td>
<td>29 (91%)</td>
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<td>15 (27%)</td>
<td>40 (73%)</td>
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<td>17 (34%)</td>
<td>29 (91%)</td>
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<td></td>
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<td>4 (17%)</td>
<td>29 (91%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 (34%)</td>
<td>29 (91%)</td>
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</tbody>
</table>

*Outside HindIII.
†Within HindIII.
‡Downstream of the Sma I site, and in most or all cases, probably also downstream of the P1 and P2 promoters.
§Two additional tumors had breakpoints within the Pvu II-Pvu I fragment, but a more precise breakpoint (ie, downstream or upstream of the Sma 1 site) was not determined.

Further analysis of 9 of the 11 sBL samples with a breakpoint in the Pvu II–Pvu II fragment showed that 3 had breakpoints between the 5’ Pvu II site and the Sma I site immediately upstream of P1, and 6 had breakpoints downstream of this site. Thus, at least 18 of the 32 sBLs (56%) had breakpoints downstream of the 5’ Sma I site. It is likely that most or all of these are also downstream of the c-myc promoters, P1 and P2. In contrast, only 4 (17%) of the eBLs had breakpoints downstream of the Sma I site (P < .004).

Among the 20 tumors with a breakpoint outside the HindIII fragment encompassing c-myc, 17 of which were eBL, 12 were associated with a rearranged Pvu II–Pvu II fragment of 1.7 kb, indicating mutation of the Pvu II site in the first exon (Table 1).

Chromosome 14 Breakpoints

For the purposes of this analysis we divided the chromosome 14 breakpoints into two major regions: within or outside the Sμ region (as defined in Materials and Methods) (Fig 4). Non-Sμ breakpoints were more than twice as frequent in the overall series as Sμ breakpoints (40 of 55 [73%] compared with 15 of 55 [27%]). Sμ breakpoints were slightly more frequent in sBL than in eBL. Sμ breakpoints occurred in 10 of 32 (31%) sBLs and 5 of 23 (22%) eBLs (Fig 4). This difference was not statistically significant.

Correlation Between Breakpoints on Chromosomes 8 and 14

Sμ breakpoints were associated twice as frequently with tumors in which the HindIII fragment was rearranged (12 of 35 [34%]; 9 of 29 sBLs and 3 of 6 eBLs) than in tumors with a breakpoint outside the c-myc HindIII fragment (3 of 20 [15%]) (Fig 5). This difference was not statistically significant. In fact, the majority of the Sμ breakpoints (12 of 15 [80%]) occurred in tumors with a rearranged c-myc gene.

Among the eBL specimens, the most frequent combination of breakpoint locations was a breakpoint outside the HindIII fragment on chromosome 8 and outside the Sμ region on chromosome 14 (some of these tumors could have had breakpoints on chromosomes 2 or 22). Fifteen of the 23 tumors (65%) fell into this category (Table 3). Only 3 of 23 eBLs (13%) had a breakpoint within the HindIII fragment (ie, a rearranged c-myc gene) and a Sμ breakpoint. In contrast, only 2 of 32 sBLs (6%) had chromosome 8 breakpoints outside c-myc and non-Sμ breakpoints on chromosome 14 (significantly different from eBL; P < .00001), whereas 9 of 32 (28%) had both a rearranged c-myc and an Sμ breakpoint. The majority of the sporadic tumors, however, had a rearranged c-myc gene and a nonswitch breakpoint (20 of 32 [63%]). All of these tumors

Fig 3. Histogram showing breakpoint location on chromosome 8 in relation to geographic origin. Regions on chromosome 8 are shown in the schematic below. H, HindIII; Ps, Pst I; Pv, Pvu II.
are likely to have had 8;14 translocations, because all of the rearranged c-myc genes had breakpoints within the 5' region of the gene. Breakpoints in this region of c-myc have not been described in association with variant translocations.

Correlation Between Breakpoints and EBV Association

EBV DNA was present in 100% (23 of 23) of the eBLs tested in this series and 38% (12 of 32) of the sBLs \((P < .00001)\). When the EBV association of only fresh tumors was examined, 9 of 20 sBLs (45%) were EBV positive.

EBV DNA was present in 19 of 20 samples (95%) with a chromosome 8 breakpoint outside c-myc (Fig 6). Among the samples with breakpoints within the HindIII fragment encompassing c-myc, there were roughly equal numbers that were positive for EBV (16 of 35 [46%]) and negative for EBV (19 of 35 [54%]) (Fig 6). The difference between EBV association in tumors with and without a rearranged c-myc gene was statistically significant \((P < .00053)\).

We also observed a correlation with respect to chromosome 8 breakpoints near the P1 promoter region. All three tumors with a breakpoint within the \(Pvu\ II-Sma\ I\) region immediately upstream of the first c-myc exon were EBV negative, as were an additional four tumors with breakpoints between the \(Pst\ I\) and \(Pvu\ II\) sites; i.e., seven of seven tumors with breakpoints immediately upstream of exon 1 (between the 5' \(Pst\ I\) site and \(Sma\ I\)) were EBV negative. All of these tumors were of sporadic origin. In contrast, 6 of 9 tumors with breakpoints within the \(Sma\ I-Pvu\ II\) region...
Table 3. Breakpoint Combinations in Endemic and Sporadic Burkitt's Lymphomas

<table>
<thead>
<tr>
<th></th>
<th>R/Sp</th>
<th>R/Non-Sp</th>
<th>U/Sp</th>
<th>U/Non-Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>eBL (23)</td>
<td>3 (13%)</td>
<td>3 (13%)</td>
<td>2 (9%)</td>
<td>15 (65%)</td>
</tr>
<tr>
<td>sBL (32)</td>
<td>9 (28%)</td>
<td>20 (63%)</td>
<td>1 (3%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Total (55)</td>
<td>12 (22%)</td>
<td>23 (42%)</td>
<td>3 (5%)</td>
<td>17 (31%)</td>
</tr>
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</table>

Abbreviations: R, rearranged c-myc; U, unrearranged c-myc; Sp, break within the switch μ region; non-Sp, break outside the switch μ region.

(first exon) were EBV positive and 7 of 12 tumors with first intron breakpoints were EBV positive.

Tumors that had chromosome 14 breakpoints within the Sp region were equally divided between EBV positive (eight) and negative (seven). In those that had non-Sp breakpoint locations, 68% (27 of 40) were EBV positive. No clear pattern emerged with regard to EBV association with combinations of breakpoint locations on chromosomes 8 and 14, apart from the finding that all tumors with breakpoints outside c-myc and outside Sp were EBV positive (Table 1).

DISCUSSION

We have previously demonstrated an association between chromosome 8 breakpoints, identified at the level of restriction enzyme fragments, and the geographic origin of BL. These data have now been confirmed in a larger series examined in more detail, and additional observations have been made regarding the breakpoint on chromosome 14, associations between chromosome 8 and 14 breakpoints, and associations between the breakpoint location and the presence of EBV DNA in the tumor cells. We have confirmed that the major correlate of geography is the breakpoint on chromosome 8. In sBL with 8;14 translocations a chromosomal breakpoint far 5' (ie, outside the HindIII and usually the EcoRI sites 5' of c-myc) is uncommon, but this was the most frequent breakpoint location in eBL. In contrast, we could discern no clear-cut correlation between chromosome 14 breakpoints and geography in this series of cases, although Sp breakpoints were particularly uncommon when the breakpoint was outside c-myc on chromosome 8, a type of translocation predominantly associated with endemic tumors. In fact, 80% of Sp breakpoints were associated with a rearrangement of c-myc.

The possibility that the frequency of breakpoints within other heavy-chain switch regions is associated with geographic origin has not been addressed by the present study.

The most likely explanation for the presence of the associations between breakpoint location and geography is that the endemic and sporadic forms of this disease that bear 8;14 translocations differ pathogenetically, probably as a consequence of differences in the cell type that is a target for the translocational event. However, the molecular differences between eBL and sBL have a significance beyond simply providing molecular markers, because they indicate that there are differences in the mechanism whereby the c-myc gene is deregulated, the primary consequence of the translocations. Sometimes, as occurs predominantly in eBL, the major portion of the c-myc regulatory region remains intact, although in such tumors mutations are always present in c-myc. In other tumors (the majority of sBL), a large part of the regulatory region is separated from the gene, and in many cases (where the breakpoint is downstream of the normal promoters, P1 and P2), transcription is initiated at sites within the first intron. The breakpoint on chromosome 8 also determines the distance between c-myc and immunoglobulin sequences, which on average is considerably greater in endemic tumors.

However, the breakpoint on chromosome 8 should not be considered in isolation. The breakpoint region on chromosome 14 determines which regions of the immunoglobulin gene locus (which includes several enhancer regions capable of influencing the expression of c-myc) will be juxtaposed to c-myc. The recent recognition of an enhancer...
element downstream of the heavy-chain locus in rats and the high probability that a similar enhancer exists in humans permit the conclusion that 8;14 translocations result in at least one immunoglobulin-enhancer element being present on the same chromosome as c-myc, even though the proximity of such enhancer elements to the c-myc promoters (including elements in the first intron of the gene capable of acting as promoters) varies. Our previous analysis, based on small numbers of cases, suggested that the breakpoint location on chromosome 14 also differed between endemic and sporadic tumors. The present, larger series indicates that although Sμ breakpoints are somewhat more frequent in sBL, breakpoints outside Sμ predominate in both endemic and sporadic tumors. Nonetheless, the chromosome 14 breakpoint may well define different subtypes of BL when considered in the context of the chromosome 8 breakpoint (see next section).

If the breakpoint locations are even partly determined by such factors as the precise location of DNA binding proteins on the DNA strand, it is likely that they relate to the state of differentiation of the cell in which the translocation occurred. This appears particularly likely in the case of the chromosome 14 breakpoints, because sequential rearrangements within the immunoglobulin locus occur as part of the normal differentiation of B cells. If the chromosomal translocation does reflect the state of differentiation of the cell at the time of the translocation, it is also possible that the breakpoint locations could be relevant to the clinical features of BL. For example, the production or response to different growth factors could differ in subtypes of the tumor, thus influencing the anatomic regions most often involved. Marked differences in the clinical characteristics between eBL and sBL have, in fact, been observed.

When examining the present data, it should be taken into account that cytogenetic analysis was not available in all tumors. Therefore, in some cases (ie, those with unarranged c-myc genes and a breakpoint outside Sμ) we cannot exclude the presence of a variant translocation. Variant translocations, however, are known to constitute a small proportion (approximately 20%) of both sporadic and endemic tumors, and certain breakpoint locations (eg, a c-myc gene rearranged because of a breakpoint in its 5′ region) have not been described in association with a variant translocation, whereas the demonstration of a breakpoint within Sμ or Jμ confirms the presence of an 8;14 translocation. Thus, variant translocations, if they occurred in this series, are almost certainly confined to a small subset of the endemic tumors.

**Associations Between Breakpoints on Chromosomes 8 and 14**

Our data showed several intriguing associations between the breakpoint regions on chromosomes 8 and 14. The most common breakpoint combination in eBL, which occurred in 65% of tumors, was a breakpoint outside the HindIII fragment encompassing c-myc on chromosome 8, associated with a non-Sμ breakpoint (ie, outside the HindIII fragment encompassing Sμ) on chromosome 14 (Table 3). This pattern occurred in only 6% of sporadic tumors. The most frequent combination of breakpoints in sBL, which occurred in 63% of cases, was a breakpoint within the HindIII fragment encompassing c-myc, associated with a nonswitch breakpoint on chromosome 14. Breakpoints within the HindIII fragment on chromosome 8 and the Sμ region on chromosome 14 occurred in 28% of sporadic tumors and 13% of endemic tumors.

These findings further support the probability that there are biological and etiological differences between sporadic and endemic forms of BL, and that the mechanism of deregulation of c-myc differs in the major subtypes of sBL and eBL. In this regard, a particularly striking finding was that at least 56% of sBLs have breakpoints downstream of the 5′ Sma I site. Many of these breakpoints are downstream of the normal c-myc promoters (P1 and P2), such that the translocation of the translocated gene must be initiated in the first c-myc intron. This is presumably made possible by the juxtaposition of immunoglobulin gene enhancer elements, because such truncated c-myc genes do not appear to be functional in the absence of heterologous enhancer sequences coupled in cis. Tumors of this kind were observed in only 17% of eBLs.

In endemic tumors, when the breakpoint is far 5′ of c-myc such that the gene is grossly intact and transcription is initiated from P1 and P2, albeit in a different ratio from normal cells, mutations can invariably be found in a 300-bp region encompassing the first exon/intron boundary of c-myc (RD-F, unpublished observations). Such mutations often involve the PvuII site in the first exon, as observed in 60% of the tumors in this chromosome 8 breakpoint category in the present series. While such mutations may result in c-myc deregulation via abrogation of the block to message elongation present at the 3′ end of the first exon, a recognized method of regulation of c-myc transcription, or of absence of the larger of the two major c-myc encoded proteins (67 Kd), these mutations are not in themselves sufficient to cause deregulation of c-myc (R. Dalla-Favera, unpublished observations).

Breakpoints within the 5′ flanking sequences of c-myc could influence c-myc expression by a separate mechanism. The immediate 5′ flanking sequences and the 5′ region of the c-myc gene itself are known to incorporate elements involved in the regulation of transcription from P1 and P2. We have previously described one cell line in which a breakpoint immediately 5′ of c-myc results in transcription only from the P2 promoter, presumably because of structural changes occurring in the regulatory elements immediately adjacent to the breakpoint. It is likely, therefore, that disruption of the enhancer/suppressor elements for P1 and P2 is the primary result of breakpoints in the 5′ flanking region of c-myc.

There are several considerations with respect to the significance of the breakpoint locations in the heavy-chain region on chromosome 14. Breaks within the Sμ region, or downstream of Sμ (eg, in another switch region) result in translocation of the Ig enhancer situated between the J and Sμ regions on chromosome 14, to chromosome 8, where it
cannot influence the expression of the translocated c-myc gene (which has simultaneously been translocated to chromosome 14). It is of interest that in 80% of such tumors, the c-myc gene was rearranged, a finding that is consistent with the idea that greater disruption of the c-myc regulatory region is required when the 5' Ig enhancer is not available to influence c-myc expression. Breakpoints close to, within, or upstream of the Jμ region (which result in the heavy-chain enhancer remaining on chromosome 14 and, almost certainly, influencing expression of the translocated c-myc gene to which it is juxtaposed) occurred in association with all chromosome 8 breakpoint locations. Whether the breakpoint lies in the Jn or Sp regions on chromosome 14, the presumptive enhancer 3' of the heavy chain region lies adjacent to the translocated c-myc gene, and although these enhancer elements may lie more than 100 kb away from c-myc, it is highly probable that they, and/or other elements within the juxtaposed Ig locus, influence the expression of the structurally altered c-myc gene. Whether there are biological differences that relate to the specific enhancer used is not known.

Associations Between Chromosome Breakpoint Locations and Presence of EBV

The presence of EBV in most eBLs but in a minority of sBLs has led to conceptual difficulties in establishing a role for the virus in the pathogenesis of BL. By examining a larger number of samples, a pattern begins to emerge that may shed some light on the presumptive role of EBV in tumorigenesis. Ninety-five percent of tumors with breakpoints outside c-myc were EBV positive, those with immediate 5' breakpoints were invariably EBV negative (seven of seven), whereas those with breakpoints downstream of the Sp1 site upstream of exon 1 were as frequently EBV positive as EBV negative. Although this association is at present tentative (eg, particular breakpoint locations and EBV association both may be more likely in the presence of another factor[s] and thus may be independent of each other), and more precise localization of the breakpoints on chromosome 8 is required, these data raise the possibility that EBV may play an important pathogenetic role in a fraction of tumors defined by the abnormalities in the regulatory region, predominantly a consequence of the breakpoint location. This implies, in turn, that one or more EBV-latent genes, most probably EBNA 1, because it is the only gene invariably expressed in BL and is also a transcriptional activator, may positively interact, directly or indirectly, with regulatory regions that remain associated with the translocated c-myc gene. If this were so, EBNA 1 could contribute to deregulation of the gene, or even, by binding to specific sequences of c-myc, influence the breakpoint location. Specific alterations in the regulatory region, whether deletions or mutations, may permit or increase this effect, which is functionally similar to the enhancement brought about by juxtaposed Ig sequences.

In EBV-negative tumors, the influence of an EBV gene is not required, either by virtue of the specific structural changes that have occurred in c-myc or because alternative genetic elements (including the possible participation of another, as yet unidentified, virus) replace the putative EBV-mediated effect.

Classification of BL by Molecular Characteristics

When the largest group of eBL (a breakpoint on chromosome 8 outside the c-myc gene associated with a non-Sp breakpoint on chromosome 14) is removed from consideration, the pattern of chromosomes 14 and 8 breakpoints in the remaining endemic tumors is similar to that of the sporadic tumors, although the total numbers are small. Thus, the BL subtype associated with breakpoints outside c-myc and Sp may be the only type of tumor to which Equatorial Africans are predisposed. It will be of interest to determine whether this subtype is characteristically associated with jaw tumors, and with EBV, in other world regions.

Finally, our findings indicate that the traditional subtypes of BL (eBL and sBL) are not homogeneous, and that these terms might better be replaced by designations based on the chromosomal breakpoint locations and EBV association. A preliminary classification of this kind has been published elsewhere.

REFERENCES


Patterns of chromosomal breakpoint locations in Burkitt's lymphoma: relevance to geography and Epstein-Barr virus association

B Shiramizu, F Barriga, J Neequaye, A Jafri, R Dalla-Favera, A Neri, M Gutierrez, P Levine and I Magrath