Histogenetic Correlations Between Subcategories of Small Noncleaved Cell Lymphomas

By Takahiro Yano, Johan H.J.M. van Krieken, Ian T. Magrath, Dan L. Longo, Elaine S. Jaffe, and Mark Raffeld

To assess the biologic relevance of the morphologic distinctions between subtypes of small noncleaved cell lymphomas (SNCL), i.e., the sporadic Burkitt’s type (sBT) and the non-Burkitt’s type (nBT), we have examined the molecular organization of several lymphomagenic oncogenes (c-myc, bcl-1, bcl-2) and the potential pathogenetic contribution of the Epstein-Barr virus (EBV). Twenty-nine cases of SNCL, not associated with immunodeficiency syndromes, were reviewed and classified as sBT (18 cases) or nBT (11 cases) without knowledge of the clinical or molecular data. Southern blot analysis of 18 sBTs found 17 to contain c-myc rearrangements. Fifteen of these comigrated with an Ig heavy-chain gene segment, indicating t(8;14) translocation. Chromosome 8 breakpoints were clustered in the first exon and the first intron of the c-myc gene. Chromosome 14 breakpoints mapped to the JH locus in three tumors, the Sp locus in nine tumors, and the Sx locus in the remaining three tumors. Cases involving the Sp locus appeared to have a more rapid clinical course. All sBTs possessed germline bcl-2 and bcl-1 gene fragments. In contrast, Southern blot analysis of 11 nBTs found none with c-myc rearrangements. Rather, three of 10 evaluable nBTs had bcl-2 rearrangements. The remaining seven showed no evidence of involvement by any of the lymphoma-associated oncogene/breakpoint regions studied. EBV genome was detected in two sBTs and in one nBT, and thus was not a distinguishing feature. These results indicate that the subtle histologic differences that distinguish subcategories of SNCL are significant biologically and reflect distinct molecular mechanisms of lymphomagenesis. Furthermore, the data suggest that the nBTs comprise a heterogeneous group with respect to their molecular genetic composition and confirm the remarkable molecular genetic homogeneity of the sBT group.

This is a US government work. There are no restrictions on its use.

THE WORKING FORMULATION of non-Hodgkin’s lymphomas recognizes two morphologic subcategories of small noncleaved cell lymphomas (SNCL), the Burkitt’s type (BT) and the non-Burkitt’s type (nBT).1 The BT was defined according to criteria delineated by the World Health Organization (WHO)-sponsored study of 1969,2 while the nBT was defined as a diffuse high-grade lymphoma closely resembling the BT, but not fully meeting the strict morphologic criteria prescribed by the WHO. The BT can be further subdivided, by clinical and epidemiologic criteria, into the endemic type (eBT), which primarily occurs in geographically restricted regions in Africa, and the sporadic type (sBT), which, as the name suggests, occurs sporadically throughout the world.

eBTs, a well-recognized clinicopathologic entity, are easily separable from nBTs by virtue of their unique clinical and epidemiologic features. sBTs, on the other hand, while sharing some clinicopathologic features with their endemic cousins, are less stereotypic and may show significant clinical, pathologic, and immunologic overlap with the more heterogeneous nBT group.3,8 Although some clinical distinctions between these latter two subtypes have been noted, particularly with regard to age distribution and to a lesser extent the anatomic distribution of disease at presentation, the clinical significance and biologic relevance of separating the sBT from nBT have remained controversial.3,8

Further complicating this issue are questions raised concerning the accuracy and reproducibility of the pathologic diagnosis. Recognition of the subtle histologic differences between sBT and nBT is difficult at best and requires optimally preserved tissue and adequate material for review. The low concordance rate reported even among expert hematopathologists in distinguishing sBTs from nBTs has added fuel to the controversy.

Over the past decade, it has become clear that many tumors are characterized by specific molecular abnormalities that often involve important cellular genes thought to have a role in growth control. In addition to their obvious biologic importance, these molecular abnormalities can be exploited to aid in the identification of biologically disparate entities, which may otherwise be difficult to distinguish from one another.

The Burkitt’s lymphomas (both the sBT and eBT) are characterized by specific chromosomal translocations that juxtapose areas within or near the c-myc proto-oncogene locus on chromosome 8 to an Ig gene locus on chromosome 2, 14, or 22.9,10 The resulting deregulation of the c-myc gene has been implicated in tumorigenesis.11 Although both the sBT and the eBT SNCLs have been the focus of intense molecular analysis, few of the nBT have been carefully studied, leaving considerable uncertainty as to whether distinct molecular genetic features characterize this subtype as well. Occasional cases with the t(14;18) translocation, and/or its molecular counterpart, bcl-2 rearrangement, have been reported,12,15 as have rare cases showing t(8;14) and/or its molecular counterpart, c-myc gene rearrangement.16 However, these reported cases are few, and generally the criteria for both case selection and pathologic
diagnosis are not defined. Furthermore, there are no studies that directly compare the molecular involvement of known lymphoma-associated oncogenes (and other potentially important cofactors such as Epstein-Barr virus [EBV]) in carefully selected cases of sBT and nBT. We therefore wished to assess the biologic significance, as well as the pathologic relevance, of separating sBT from nBT by comparing the molecular organization of lymphoma-associated chromosomal loci (c-myc, bcl-1, and bcl-2) and the possible pathogenetic contribution of EBV in these morphologically and clinically similar high-grade lymphomas.

**MATERIALS AND METHODS**

**Histopathologic subcategorization.** Twenty-nine cases of SNCL were selected for this study on the basis of availability of frozen tissue for the subsequent molecular biologic analysis. Although the requirement for frozen tissue potentially introduces a selection bias, all patients on lymphoma protocols at this institution are required to have an in-house biopsy as part of their initial staging evaluation (with the exception of those requiring immediate therapy). Excluded from this study were all cases arising in a background of either congenital or acquired immunodeficiency syndromes, as the pathogenesis of disease in these settings may not be comparable. Approval was obtained from the Institutional Review Board for these studies. The cases were classified as either sBT or nBT by one of us (E.S.J.), after review of formalin- or B5-fixed sections stained with hematoxylin and eosin. Classification was performed without knowledge of the clinical or molecular data. Diagnostic criteria as defined by WHO were used for sBT. Briefly, cases were subclassified as sBT if the tumors were composed of a diffuse and monotonous population of neoplastic cells with a narrow rim of distinctly amphophilic cytoplasm and a round to oval nucleus that contained multiple small but distinct basophilic nucleoli and coarse chromatin (Fig 1A). nBTs tended to have a more finely dispersed chromatin pattern and sometimes a single prominent, centrally positioned nucleolus (Fig 1B). nBT often had a more polymorphous composition, with more numerous smaller lymphoid cells admixed with the larger transformed cells.

**Clinical data.** Clinical data concerning age, sex, stage of disease, and sites of presentation were tabulated for each lymphoma subgroup to verify that our cases of SNCL were representative of other cases reported with the above histologies. Because patients were selected for this study on the basis of availability of frozen tissue, a potential selection bias has been introduced as discussed above. However, there are no significant differences in the clinical parameters examined between patients in this study and other patients with the same histologic subtypes of lymphomas treated in this institution.

**Immunohistochemistry for surface IgS.** Neoplastic cells from ascitic fluid, solid tumors, or biopsies of involved lymph nodes were prepared and stained with fluoresceinated polyclonal antisera against human IgG, IgA, IgM, κ, λ, or with monoclonal antibodies anti-γ, anti-α1, anti-α2, anti-δ, anti-κ, and anti-λ IgS (Becton Dickinson, Monoclonal Antibodies, Mountain View, CA) and anti-μ Ig (Bethesda Research Laboratories, Gaithersburg, MD) for analysis by flow cytometry and immunohistochemistry as described.

**Molecular analysis: DNA extraction and Southern blot hybridization.** High molecular weight DNA was isolated directly from involved frozen tissue samples or cell suspensions as described previously. Following restriction enzyme digestion with HindIII, EcoRI, Bam HI, or PvuII (Bethesda Research Laboratories), the DNA was size-fractionated by agarose gel electrophoresis and transferred to nylon membranes (Gene Screen Plus, New England Nuclear Research Products, Boston, MA). The filters were serially hybridized with 32P-labeled probes for 18 to 24 hours and washed under stringent conditions according to the instructions of the supplier. Autoradiographs using Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) were developed after 1 to 7 days.

**DNA probes: Oncogene and viral probes.** The three different probes used to assess the configuration of the c-myc gene are shown (see Fig 3). The third exon probe, a 1.4-kb ClaI/EcoRI fragment (gift of Dr F. Wong-Staal), was used for screening of c-myc rearrangements. Both the 5’ first exon probe, a 1.1-kb HindIII/ClaI fragment, and the first exon probe, a 0.9-kb PvuII fragment (both provided by Dr D. Levens), were used for mapping chromosome 8 breakpoints in cases with c-myc rearrangements. The latter probe was also used to detect potential PvuII site mutations in the first exon (discussed below).

Fig 1. Representative histologies of SNCL. (A) Burkitt’s type. (H&E, B5 fixative; original magnification × 330.) (B) Non-Burkitt’s type. (H&E, B5 fixative; original magnification × 330.) The nuclei of the Burkitt’s type are uniform in size and shape with coarsely clumped chromatin and several small basophilic nucleoli. By comparison, nuclei of the non-Burkitt’s type show increased pleomorphism, with more finely dispersed chromatin and more prominent, sometimes eosinophilic, nucleoli.
Other oncogene probes used included *bcl-1*, a 2.1-kb *SalI* fragment (gift of Dr. Y. Tsujimoto);22 *bcl-2* (mbr), a 2.8-kb HindIII/*EcoRI* fragment (gift of Dr. S.J. Korsmeyer);23 *pFL-2* (mer), a 4.0-kb *EcoRI* fragment (gift of Dr. M.L. Cleary);24 and *pB16* (5' *bcl-2*), a 1.6-kb *EcoRI* fragment (gift of Dr. Y. Tsujimoto).25 Rearranged bands were examined for possible comigration with an immunoglobulin heavy-chain gene fragment.

Two EBV terminal repeat probes, a 1.9-kb *XhoI* fragment and a 2.8-kb *EcoRI* I fragment (kindly provided by Dr. N. Raab-Traub), were used to assess not only the presence of the virus, but also clonality of the tumors, dominant forms of the viral genome, and the replicative status of the virus present in the tumor cells.26

Ig gene probes. The following DNA probes were used to investigate the molecular organization of the Ig genes and for comigration studies in cases with *c-myc* or *bcl-2* region rearrangement as indicated: *JH* (3.8-kb *BglII* fragment), *Jk* (1.8-kb *SalI* fragment), *Cc* (2.5-kb *EcoRI* fragment), *Ca* (0.8-kb *EcoRI* fragment), *Sµ* (2.0-kb *SalI* fragment), *Cµ* (1.3-kb *EcoRI* fragment), *Cα1* (0.9-kb *PstI* fragment), *Cy4* (6.6-kb *BamHI/HindIII* fragment), and *Cε* (2.6-kb *BamHI* fragment).26,27 All were kindly supplied by Dr. P. Leder, except *Jk* probe, a gift from Dr. S.J. Korsmeyer, and the *Ca1* and *Cy4* probes, provided by Dr. K. Kelly.

**Mapping of chromosome 8 breakpoints.** The location of chromosome 8 breakpoints was mapped by sequential hybridizations using the three *c-myc* probes previously described (see Fig. 3). If the rearranged bands detected with any two of the *myc* gene probes differed in size in the same *HindIII* or *EcoRI* restriction enzyme digest, the breakpoint was considered to lie between the two probes (see Fig. 3). A breakpoint was considered to lie inside a probe fragment if two *c-myc* rearrangements were identified in two or more of the digests.

**Evaluation of *PvuII* site mutations in the first exon of the *c-myc* gene.** In cases with chromosome 8 breakpoints occurring far 5' or 3' to the *c-myc* locus, it is not possible to detect breakpoints directly using conventional probes. However, since up to 60% of these lymphomas have been reported to have mutations in the germline *PvuII* site located near the 3' boundary of the first exon,28,29 it is possible to look indirectly for evidence of *myc* region rearrangement by evaluating the status of this *PvuII* site. For this purpose, *PvuII*-digested DNA was probed with our first exon probe. Cases were scored positive for *PvuII* site mutations if a novel 1.8-kb fragment, predicted by the loss of the mutated *PvuII* site and extension to the next 3' *PvuII* site, appeared with a corresponding decrease in intensity of the normal 0.9-kb germline fragment.

**Assignment of the breakpoints in the Ig gene loci.** Breakpoints in the *JH* locus were identified by comigration of a *JH* fragment with the appropriate enzyme digests. Comigration of the *Jos* fragment with a *c-myc* fragment indicates the break has occurred within or near one of the several switch loci including *Sµ*, *Sa*, or *Se*, and, under certain conditions, *Sγ*, since the *Sα* probe strongly cross-hybridizes *Se* and *Se*, but shows only limited homology to *Sγ* sequences.22 Additional comigration studies using specific constant region probes (Cµ, Ca, Cy, or Cε) with the appropriate enzyme digests showed which switch loci were involved in the translocations. In those cases where we could not demonstrate comigration with an Ig heavy-chain gene fragment, potential light-chain gene comigration was studied using the appropriate enzyme and probe combinations.

**RESULTS**

**Histopathologic subcategorization and clinical observation.** Eighteen patients were diagnosed as sBT, while 11 were subclassified as nBT. By strict adherence to the morphologic criteria, the majority of the patients could be readily subcategorized. Review diagnosis was coincidental with the initial histopathologic diagnosis, except in one patient who had been diagnosed as "diffuse undifferentiated lymphoma, not otherwise classified" initially, and was reviewed as Burkitt's lymphoma.

Table 1 summarizes pertinent clinical information characterizing each of our subgroups of SNCLs. The median age of the 18 patients diagnosed with the sBT was 20 years and ranged between 3 to 37. Nine patients were in the pediatric age group, while nine were considered adult (defined as >18 years). There were 10 males and eight females. Of the 17 patients on whom full clinical information was available, 13 (76%) presented with advanced-stage disease, while four (24%) presented with early-stage disease. All 17 patients presented with extranodal involvement, and all but one of these had gastrointestinal involvement. The reproductive organs were the second most frequent site of involvement at presentation and this occurrence was more common in females. Only one patient had peripheral lymph node involvement (in addition to abdominal disease) at presentation.

Of the 11 nBT SNCLs studied, 10 occurred in adults; only one patient fell into the pediatric age group. The median age was 35 years and ranged between 8 and 75. There was a preponderance for this disease to occur in males, with a male to female ratio of 9:2. Eight patients (73%) presented with advanced-stage disease, while three (27%) had early-stage disease. Five of the 11 patients with the nBT presented with peripheral lymph node involvement only. Six of the patients had involvement of extranodal sites at presentation and, of these, three showed peripheral lymph node involvement in addition.

The above profiles of clinical features are typical of other studies of these lymphoma subtypes reported in the literature.24

![Table 1. Clinical Data of SNCLs](image-url)
### Table 2. Immunologic and Molecular Data of sBT

<table>
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<th>Case</th>
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<th>Ig Genes</th>
<th>PvuII Mutation</th>
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Abbreviations: R, rearranged; G, germline; D, deleted; NI, not interpretable; ND, not determined; +c, clonal EBV present.

*Summary of three breakpoint regions.

†Chromosome 8 breakpoints as defined in Fig 3.

**Immunohistochemistry for surface Iggs.** All 17 sBTs examined expressed surface IgM (Table 2) in complete accordance with the molecular finding of at least one intact JH-Sp-Cp recombination (data not shown). nBTs also uniformly expressed surface IgM, except for one case that expressed IgG (Table 3). With respect to light chains, K was expressed in 11 sBTs and in four nBTs; λ was expressed in four sBTs and four nBTs.

**Molecular analysis: Burkitt's type.** All sBTs showed JH rearrangements and Jκ rearrangements or deletions (Table 2). Jλ rearrangements were seen in five of 18 cases examined (27%). A c-myc rearrangement was detected in 17 cases (94%) (Fig 2A). (Although cases B10 and B15 appear to be germline in the HindIII digest, both showed rearrangements in the BamHI and EcoRI digests [data not shown], suggesting that in the HindIII digest, comigration of a rearranged band with the germline band occurred.) Sequential hybridization of the blots with the 5' first exon probe, the first exon probe, and the third exon probe demonstrated chromosome 8 breakpoints to lie between the 5' HindIII and first PvuII site (region 1) in four tumors (24%) (HindIII-ClaI in one, ClaI-PvuII in three), between the first and second PvuII sites (region 2) in four other tumors (24%), and between the second PvuII site and the second ClaI site (region 3) in the remaining nine tumors (53%) (Fig 3). PvuII site mutations in the first exon of the c-myc gene were seen in two tumors (cases B11 and B17). No involvement of either the bcl-2 locus (using three different probes) or the bcl-1 region was detected. Clonal EBV DNA was present in two tumors (B11, B17) in the latent, episomal form (Fig 4). There were no other obvious clinical, immunologic, or molecular differences between these latter two cases and the other cases of sBT.

Comigration studies showed that 15 sBTs had c-myc

### Table 3. Immunologic and Molecular Data of nBT

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<th>Case</th>
<th>Surface Ig</th>
<th>Ig Genes</th>
<th>PvuII Mutation</th>
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Abbreviations: R, rearranged; G, germline; D, deleted; Ni, not interpretable; ND, not determined; +c, clonal EBV present.

*Summary of three breakpoint regions.
Fig 2. Southern blot analysis of SNCLs. (A) sBT cases, HindIII digest probed with the c-myc third exon probe. All but cases B10, B14, and B15 demonstrate c-myc rearrangements. However, B10 and B15 show distinct rearrangements in the BamHI and EcoRI enzyme digests (data not shown). (B1) nBT cases, HindIII digest probed with the c-myc third exon probe. No case displays c-myc rearrangement. (Faint band located above germline in case NB4 represents a partial digestion product occasionally seen in the HindIII digest. EcoRI and BamHI digests of the same case show only germline bands.) (B2) nBT cases, HindIII digest probed with the bcl-2 mbr probe. Three cases show rearrangements.

Fig 3. Restriction map of the human c-myc locus on chromosome 8. E, EcoRI; H, HindIII; C, ClaI; P, PvuII; P*, hypermutable PvuII site. Exons are depicted by rectangular boxes. White rectangles are nontranslated regions; black rectangles represent coding regions. The three c-myc probes used in this study are shown immediately below the map. Locations of breakpoints in myc rearranged sBTs are shown by case number and the region (1, 2, or 3) to which each was mapped.
Fig 4. Southern blot analysis of two EBV-positive cases. Probe used is the terminal repeat probe Xhol (courtesy of Dr N. Raab-Traub); digest is BamHI. A single terminal repeat fragment is present in the two cases shown, suggesting each case contains a stably infected clonal population of cells.

EBV rearrangements that comigrated with c-myc in both HindIII and EcoRI digests. In each case, a Cε rearrangement comigrated with the 5' first exon c-myc fragment (the reciprocal rearrangement), indicating that the c-myc rearrangement occurred into a germline Sa locus. One of these tumors (case B4) was also established as the CA46 cell line in which the c-myc gene has been previously reported to be translocated to the Sa1 locus.37

Patients with Sa breakpoints appeared to have a poorer prognosis than those with Sp breakpoints, in that seven of nine patients with Sp breakpoints survived 2 or more years following diagnosis, whereas all three patients with Sa breakpoints died within 1 year of diagnosis. However, these numbers are too small to be statistically significant.

Molecular analysis: Non-Burkitt's type. Both JH and Jκ rearrangements (including deletions) were detected in all cases, except one that had only focal involvement by lymphoma histologically (Table 3). Ca rearrangements were seen in six of 10 cases (60%). Notably, we could not detect c-myc rearrangements in any of the nBTs (Fig 2B1). Furthermore, PvuII site mutations were not identified in any of these cases. However, the bcl-2 major breakpoint probe demonstrated involvement of the 18q21 locus in three cases (Fig 2B2), all of which had nodal presentation without abdominal involvement. Rearranged bands comigrated with the JH fragment, indicating t(14;18) translocation. Patients presenting with abdominal disease had neither c-myc nor bcl-2 rearrangements. Clonal EBV DNA was detected in only one case. Interestingly, this was the same case that histologically showed focal involvement by lymphoma and in which we were unable to demonstrate clonality using the conventional Ig and T-cell receptor gene probes (data not shown) (case NB7, Table 3 and Fig 4). A hcl-1 rearrangement was not seen in any tumor.

DISCUSSION

We have compared 18 cases of sBT SNCL with 11 cases of nBT SNCL with respect to the molecular configuration of several lymphoma-associated oncogenes/breakpoint regions and the presence of EBV. As expected for sBT, 17 of 18 showed c-myc rearrangement. In contrast, none of the 10 evaluable cases of nBT showed evidence of c-myc rearrangement either by direct Southern analysis or by indirect PvuII mutation analysis. Three of 10 nBTs showed bcl-2 rearrangements. We could not demonstrate involvement of other lymphoma-associated oncogenes in the remaining seven cases. EBV genome was present in a small percentage of cases of both types (two sBTs and one nBT) and was not a distinguishing feature. This study confirms the molecular genetic homogeneity of the sBT with respect to the oncogenes studied and clearly separates the majority of the nBT group from the sBTs.

Several observations regarding our molecular findings deserve further attention. In our series, three of the 17 cases of sBT with c-myc rearrangements (18%) translocated into a Sa locus on chromosome 14. Switch breakpoints other than Sp have been considered unusual and only a handful of primary cases with Sa involvement have been reported.35,38 However, previous studies have not
always attempted to discriminate between the various switch regions or may not have adequately screened for these other breakpoint locations. The location of the breakpoint in the Sa locus may be of more than academic interest. All three of our patients whose lymphomas showed a rearrangement of c-myc into Sa had aggressive clinical disease and died within 1 year of diagnosis. Similarly, Care et al. reported two patients with rapidly progressive B-cell acute lymphoblastic leukemia L3 (Burkitt’s type) with c-myc-Sa rearrangements, both of whom survived only 3 and 5 months, respectively, following diagnosis.

Sequence analysis of Burkitt’s lymphomas with breakpoints in switch regions other than Sa has shown an unusually high number of mutations in the coding exons of the c-myc gene, which presumably would be reflected at the protein level. It is possible that mutations in the coding regions of the myc gene may select for clones containing more biologically active variant myc proteins and, as a consequence, result in more aggressive tumors, thereby accounting for the apparently poor prognosis of patients carrying the Sa translocation. Analysis of additional cases and their corresponding myc sequences will be necessary to verify this possible relationship.

The lack of c-myc rearrangement in the regions close to the coding exons clearly separates the nBTs from the sBTs. While we cannot completely rule out the possibility of c-myc translocation occurring outside of the regions encompassed by our probes, such as occurs with the far 3’ breakpoints found in the eBT lymphomas or the far 3’ breakpoints found in the variant translocations, our negative findings in the PvuII mutation analysis lessen the probability that these classes of translocations have taken place.

Published data detailing the molecular genetics of the nBT group are sparse with regard to c-myc rearrangement and are also limited with regard to bcl-2. Data regarding the nBTs tend to be encompassed in larger screening studies designed to study prevalence rates of particular molecular abnormalities, in contrast to the situation for the sBTs, where the cases have generally been more carefully selected for detailed molecular genetic analysis. In these screening studies, the SNCLs are usually not further subcategorized, making it difficult to extract information specific to the nBT.

The nBTs in our study fell into two groups, those with bcl-2 rearrangements and those without detectable involvement of this oncogene. This division correlated with the anatomical distribution of disease, as bcl-2 rearrangements occurred only in patients who presented with nodal disease without evidence of extranodal involvement (three of four patients). On the other hand, patients who presented primarily with extranodal disease (with or without associated lymph node involvement) had no evidence of bcl-2 involvement. This suggests that morphologic nBT is composed of at least two distinct entities, one arising from the lymph node follicle center and related to other bcl-2 translocated tumors of nodal origin, and the other arising in extranodal lymphoid tissue and unrelated to the bcl-2 translocated lymphomas. These results are consistent with previously published data describing the lack of bcl-2 gene involvement in lymphomas arising in extranodal sites.

EBV genome, present in virtually all cases of eBT lymphoma, was identified in two of 18 cases of sBT (11%) and one case of nBT (9%). The prevalence for the sBT group is somewhat lower than previously reported figures, which have ranged from 15% to 30%. While this may simply be due to chance, there is accumulating evidence that EBV involvement in sBT varies among different geographic regions and/or in different ethnic groups. No previous prevalence figure for comparison exists for EBV involvement in nBT, with the exception of those occurring in human immunodeficiency virus (HIV)-associated lymphomas.

The only involvement of EBV in a nBT lymphoma was a case that histologically showed focal tumor involvement. Interestingly, although we could not demonstrate a clonal tumor cell population using our conventional Ig or T-cell receptor probes, the EBV terminal repeat probes easily demonstrated a clonal population. We believe that the number of tumor cells in the portion of lymph node used for the DNA extraction did not meet the percentage necessary to demonstrate clonality for single-copy genes. However, because EBV is generally present in multiple copies (up to 50 or more), the sensitivity for identifying the clonal population was amplified into the detectable range. This finding suggests that in lymphomas harboring EBV, terminal repeat segment hybridizations may be useful for the detection of early relapse or the evaluation of minimal disease.

Previous studies have focused on clinical parameters in attempting to assess the clinical and biologic relevance of distinguishing between the morphologically similar subtypes of SNCL. Although many observers have noticed differences with regard to age at presentation and to a lesser extent the distribution of disease, these studies have documented considerable clinical overlap between the nBT and sBT subtypes. As a result, the value of distinguishing nBT and sBT has remained a controversial point. However, our molecular data suggest that the majority of nBTs have a different molecular pathogenesis than sBTs. Therefore, they demonstrate that the subtle pathologic differences distinguishing sBTs from nBTs are biologically meaningful and that efforts to subclassify SNCLs should continue.

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HISTOGENETIC CORRELATIONS IN LYMPHOMAS 1289


Histogenetic correlations between subcategories of small noncleaved cell lymphomas

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