Subtypes of Epstein-Barr Virus in Human Immunodeficiency Virus-Associated Non-Hodgkin Lymphoma

By M.J. Boyle, W.A. Sewell, T.B. Sculley, A. Apolloni, J.J. Turner, C.E. Swanson, R. Penny, and D.A. Cooper

Biopsy samples obtained from 20 patients with human immunodeficiency virus (HIV)-associated non-Hodgkin lymphoma (NHL) were assessed for evidence of Epstein-Barr virus (EBV) and HIV sequences. DNA was extracted from formalin-fixed, paraffin-embedded NHL tissue and specific viral gene sequences were sought using the polymerase chain reaction (PCR). EBV sequences were found in 10 NHL samples (50%), with five tumors showing A-type and five B-type sequences. By serologic testing, 18 of 19 patients had antibodies to EBV, with 14 patients having antibodies to A-type EBV and 11 to B-type EBV. Serology confirmed the high prevalence of type B EBV in HIV-infected patients, but was not a reliable indicator of the EBV subtype present in the lymphomas. HIV sequences were present in biopsy tissue but at a level consistent with an origin from bystander HIV-infected cells. All 20 patients were negative by enzyme-linked immunosorbent assay for antibodies to human T-cell leukemia virus-type I. The high prevalence of type B EBV in these tumors is similar to the findings in endemic Burkitt's lymphoma, where 40% of the tumors have type B viral sequences. In normal populations, type B EBV is rarely found outside the nasopharynx. These studies support the hypothesis that EBV is an important cofactor in NHL in HIV-infected persons. The finding that B-type EBV is present in 25% of HIV-associated NHL suggests that this EBV subtype may be an important human pathogen with a wider geographic distribution than originally thought.

NON-HODGKIN LYMPHOMA (NHL) is a well-recognized complication of infection with the human immunodeficiency virus (HIV).1 Most lymphomas are of high-grade B-cell origin, with large cell immunoblastic lymphoma and small, noncleaved cell lymphoma dominant histologic types. The prognosis of these patients is poorer than in age-matched NHL patients not infected with HIV.2-4 Epstein-Barr virus (EBV) is a possible cofactor in HIV-associated malignancies because of its almost universal prevalence, potential immunosuppressive effects, lymphotropism, and transforming capacity.5-10 HIV itself and human T-cell leukemia virus-type I (HTLV-I) have also been considered candidates for involvement in NHL pathogenesis in these patients, as HIV is able to induce B-cell proliferation in vitro11 and HTLV-I is known to promote tumor development in other settings.12 After primary infection, all individuals retain these viruses for life.

The large number of EBV strains has recently been categorized into one of two types, based on the organization of the gene regions encoding the Epstein-Barr nuclear antigen (EBNA) 2, 3, 4, and 6 proteins.10,11 These proteins, which are expressed in EBV-transformed cells, are vital to the immortalizing function of EBV in human B cells11 and contribute to biologic differences between A-type and B-type EBV. These differences are most evident in the reduced transformation capacity of type B viruses, making the establishment of B-type EBV cell lines difficult.13

Initial results suggested the B-type virus was found predominantly in Central Africa and New Guinea.4,13 However, recent studies have found B-type virus in the throat washings of both normal and HIV-infected persons in a western population.14

This study was undertaken to assess the role of EBV and its subtypes, HIV and HTLV-I in HIV-associated NHL. Previous work has suggested that EBV is present in a significant number of HIV-associated NHL but the subtype of EBV involved is unknown.5,7 A direct pathogenic role for HIV and HTLV-I in these tumors has also been a matter of controversy.2,8,17,18

MATERIALS AND METHODS

Patient Characteristics

All patient details were collated from medical records and discussions with the treating physicians. NHL was diagnosed on histologic examination of formalin-fixed, paraffin-embedded tissue and classified using the working formulation.19 The patients represent a subset of a larger series, previously reported by this group.20 All histology was reviewed by the same histopathologist to standardize classification. Frozen or formalin-fixed tissue from the 20 patients was phenotyped for B- and T-cell specific antigens. The monoclonal antibodies (MoAbs) used included, where possible: LCA, L26, IgM, IgG, IgK, IgL, CD3, UCHT1 (Dako, Carpinteria, CA), and MB2 (Clonab, Dreieich, Germany). HIV infection was detected by multiple enzyme-linked immunosorbent assay (ELISA) tests for HIV antibodies in serum (Electro Nucleonics Inc, Columbia MD; Wellcome Diagnostics, Dartford, England; Genetic Systems Corp, Seattle, WA) and confirmed by Western Immunoblot (Bio-Rad, Richmond, CA).21 The analysis of T cells and their subsets was performed as previously described.22 For the purposes of analysis, the presence of hepatomegaly, splenomegaly, or hepatosplenomegaly defined the patient as having extranodal disease, regardless of the site from which diagnostic tissue was obtained.

Preparation of DNA

DNA was extracted from formalin-fixed, paraffin-embedded tissue. The tissue was cut out of the paraffin with a scalpel, finely...
Amplification of plasmid or viral DNA was performed using the polymerase chain reaction (PCR). Each reaction was performed in a volume of 100 μL, which contained 0.5 μg of DNA sample, 10 mmol/L TrisCl pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.01% (wt/vol) gelatin, 0.5 μg of each amplification primer (see Table 1), 200 μmol/L of each dNTP, and 2 U Taq polymerase (Tybo, Osaka, Japan). The reaction mixture was first heated for 3 minutes at 93°C and then incubated at three different temperatures using an intelligent heating block (Hybard, Teddington, UK) to amplify HIV sequences, 36 cycles of 30 seconds at 93°C, 1 minute at 50°C, and 1 minute at 70°C were used. To amplify EBV sequences, 25 cycles of 1 minute at 93°C, 2 minutes at 55°C, and 1 minute at 72°C were used. To amplify IL-5 and β-actin sequences, 25 cycles of 1 minute each at 93°C, 50°C, and 70°C were used. The amplified products were electrophoresed in 1.2% agarose gels, transferred to Hybond-N+ membranes (Amersham International, Amersham, UK) and sequences detected by DNA hybridization.

Preparation of Radiolabeled Probes

The radiolabeled EBV probes were produced by PCR amplification of each of the specific EBNA genes, using the primers shown in Table 1, with the substitution of 32P-dCTP (Amersham) for cold deoxycytidine 5'-triphosphate (dCTP) in the reaction mix. The EBNA-2A gene was obtained from the plasmid pM-BamH1,25 the EBNA-2B gene from the plasmid p-HKA7,26 and the EBNA-1 gene from the plasmid BamH1K.26 Radiolabeled probes for HIV, IL-5, and human β-actin were prepared by end-labeling synthetic DNA sequences using γ-ATP (Amersham) and polynucleotide kinase (Pharmacia, Uppsala, Sweden). These synthetic probes were designed to anneal to the PCR products at sites distinct from the primers. The sequences of the probes were for HIV: 5'- GTTTAAGGCCAAGGGAAAAAAGAAAAATA; for IL-5: 5'- TGACAGTTTCCTGTAATACC; and for β-actin: 5'- CAGCAATGTACGTTGCATTC.

Hybridization Conditions

EBV filters were prehybridized in 900 mmol/L NaCl, 90 mmol/L Na citrate pH 7, 5X Denhardt's solution, 0.1% SDS, 5 mmol/L EDTA pH 8.0, 0.1 mg/ml salmon sperm DNA, 2 mmol/L phosphate buffer pH 6.5 for 2 hours at 50°C and then hybridized overnight in the same solution with the addition of the probe. The final wash was for 30 minutes in 150 mmol/L NaCl, 15 mmol/L Na citrate pH 7, 0.1% SDS at 65°C. HIV, IL-5, and β-actin filters were prehybridized in 7% SDS, 0.25 mol/L NaPi pH 7.2, 1 mmol/L EDTA for 2 hours at 50°C and then hybridized overnight at 50°C in the same solution to which 10 mmol of radiolabeled probe had been added. The final wash was for 10 minutes at 50°C in 150 mmol/L NaCl, 15 mmol/L Na citrate pH 7, 0.1% SDS. Autoradiography was performed at ~70°C using Cronex x-ray film (DuPont, Boston, MA).

All samples were amplified and autoradiography performed for EBV genes on at least three occasions and in two separate laboratories to confirm the results and ensure reliability. However, each figure represents results obtained from a single experiment, with all samples amplified in the same PCR.

Ig gene rearrangement studies. Ig gene rearrangement studies were performed using a recently published method, which exploits primers directed toward conserved sequences of the V and J regions in a PCR.24,25 Briefly, each reaction was performed in a volume of 25 μL, which contained between 0.3 and 0.9 μg of DNA sample, 67 mmol/L TrisCl pH 8.8, 1 mmol/L 2-mercaptoethanol, 170 μg/mL bovine serum albumin, 16.6 mmol/L (NH₄)₂SO₄, 1.5 mmol/L MgCl₂, 0.2% (wt/vol) gelatin, 6.7 μmol/L EDTA, 0.25 μg of each amplification primer, 100 μmol/L of each dNTP, and 1 U Taq polymerase (Tybo). The reaction mixture was first heated for 3 minutes at 95°C and then incubated for 1 minute at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C through 40 cycles using a Gene Machine (Innovonics, Melbourne, Australia). The amplified products were electrophoresed in polyacrylamide gels, stained with ethidium bromide, and the presence of monoclonal bands (100 to 120 bp long) determined under UV illumination. Polyclonal B-cell populations are seen as a broad band of staining in this region. Assessment by restriction enzyme digestion and Southern blotting was not possible due both to the small quantities and the degraded nature of the DNA available.

Serology. Serum samples obtained from the 20 patients were tested in two ELISAs for antibodies to HTLV-I (Genetic Systems, Seattle, WA; Abbott, North Chicago, IL). Antibodies to the EBNA-1, 2A, and 2B nuclear antigens were detected using an immunoblot technique, as previously described.27 Briefly, 150 μg of protein extract from the Raji, Ijioye, and Daudi B-cell lines were electrophoresed in 5% to 15% (wt/vol) SDS-polyacrylamide slab gels and electrophoretically transferred to nitrocellulose papers. The nitrocellulose papers were then incubated with serum samples and antibodies detected with 125I-labeled protein A (New England Nuclear, Boston, MA).

Statistics. Statistical analyses were performed using the BMDP statistical package.28 Proportional-hazards model analyses were computed using program P2L of the BMDP statistical package.

RESULTS

Patients

The 20 patients were all homosexual or bisexual men with no other risk factors for HIV infection. Seven patients had high-grade, malignant lymphoma, small, noncleaved cell...
type, similar to Burkitt’s lymphoma (SNCC). Twelve patients had high-grade malignant lymphoma, large cell immunoblastic type (LCI). One patient (patient 14) had high-grade NHL by cell cytology criteria but was unable to be classified further with the available tissue. All tumors successfully phenotyped were of B-cell origin (N = 16). Four patients (nos. 8, 13, 14, and 16) were unable to be phenotyped. Patients 13, 14, and 16 had no further material clonal bands evident using the PCR technique to assess clonality (data not shown). This rate of monoclonality is similar to that found by the original investigators of this technique, who suggested that the failure to show monoclonality in all patients was due to aberrant rearrangements, such as inversions of the heavy chain locus. Using both immunohistology and gene rearrangement criteria, only patient 14 was not shown to have a B-cell tumor. Nine patients had limited nodal disease at presentation (5 = LCI; 4 = SNCC), whereas 11 had disseminated or extranodal disease at presentation (3 = SNCC; 7 = LCI). The details are given in Table 2.

Detection of EBV Sequences in NHL Biopsy Samples

To identify the presence of EBV sequences in the biopsy samples, primers complementary to regions within the EBNA-1 gene were used in the PCR. This region was chosen because every strain of EBV must contain this gene. Significant amounts of EBV DNA were detected in 10 of the biopsy samples (patients 1 through 7, 12, 17, 18) and low levels in biopsies from patients 10, 14, and 19 (detected after prolonged exposure of the autoradiograph).

Sample quality, as assessed by ethidium bromide staining of gels, showed significant and variable degradation of the DNA derived from formalin-fixed tissues. Thus, it was possible that the low level of EBV detected in the biopsy material from patients 10, 14, and 19 was due to extensive degradation in these samples. Therefore, control studies were necessary to assess and normalize each of the samples for amounts of amplifiable DNA. This was accomplished by using primers directed against the IL-5 and β-actin genes in PCR reactions. The results, shown in Fig 1, demonstrate that there was significant variability between the samples in regard to the amount of amplifiable DNA, even though 0.5 μg of DNA (as assessed by absorbance at 260 nm) was used in each PCR. Amplification of both the IL-5 and β-actin genes yielded similar results, with the biopsies of patients 5, 6, 10, and 14 showing the greatest amounts of amplifiable DNA. The discrepancy between the amounts of total DNA and PCR-amplifiable DNA reflects the degree of degradation of the DNA in these samples. Even after normalization of the samples for excessive degradation of the DNA, it was apparent that only patients 1 through 7, 12, 17, and 18 contained significant amounts of EBNA-1 gene sequence. This conclusion was supported by titration of biopsy sam-

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The patient number refers to the same patient as in other figures. Serology results are recorded as + when antibody was detected and 0 where undetected. Dashes indicate a result was not available.

Abbreviation: EN, extranodal disease.

*The patient is still alive, and the survival was calculated to September 30, 1990.
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Figure 1: Analysis of DNA from tumor biopsies by PCR. Patient samples are numbered from 1 to 20. The position of molecular weight markers (bp) is shown in the left hand column, along with bars indicating the positions of the amplified fragments. The column marked + indicates the result in positive control cell lines as follows: EBNA-1, EBNA-2A, EBNA-2B, HIV, IL-5, β-actin.

The sera of all 10 patients with EBV in their tumor tissue contained antibodies to EBV nuclear antigens 1, 2A, and 2B (Table 2). The sera of all 10 patients with EBV in their tumor tissue contained antibodies to EBV antigens. Many of these sera showed dual reactivity to EBNA-2A and -2B antigens. EBV serology did not reliably predict the subtype of EBV in the tumor, nor was it helpful in predicting which patients had EBV-related tumors, as almost all subjects showed some reactivity to EBV antigens.

The HTLV-I serology was negative by both ELISAs in all 20 patients. Therefore, this retrovirus is unlikely to contribute to the pathogenesis of HIV-associated NHL.

Detection of HIV Sequences in NHL Biopsy Samples

HIV sequences could be found in nine of the patient samples after prolonged exposure of the autoradiograph (Fig 1). The patient samples were compared with dilutions of DNA from an HIV-infected cell line (Hut PW) amplified under the same conditions. Fifty picograms of cell line DNA was able to produce a signal equivalent to 500 nanograms of the strongest responding tumor-derived DNA (patient 12; Fig 1), a dilution of 1 in 10,000 (Fig 3). Again, the biopsies contained predominantly but not exclusively NHL tissue. Thus, the results of these titering experiments, though not strictly quantitative, suggest that the source of these sequences is HIV infection of surrounding, nonmalignant cells, which showed approximately 100- to 1,000-fold less EBNA-1 sequence in the biopsy from patient 19 (the patient with the strongest of the low positive results) as compared with two other positive samples (patients 3 and 7) and patients 2 and 4, who had the weakest signals of the patients thought to contain significant amounts of EBV gene sequence (Fig 2). As the biopsies contained predominantly but not exclusively NHL tissue, the titering technique used, though not strictly quantitative, suggests that the low levels of EBV sequences in the biopsies of patients 10, 14, and 19 probably reflect EBV infection of surrounding, nonmalignant cells. Indeed, the biopsy from patient 10 was from the oropharynx, a tissue from which EBV is regularly isolated.

Only one type of EBNA-2 gene was found in each of the 10 patients in which EBNA-1 sequences were detected. Five patients (nos. 2, 4, 5, 6, and 17) had tumors in which the EBNA-2A gene was found and five patients (nos. 1, 3, 7, 12, and 18) had the EBNA-2B gene sequence demonstrable. All those patients negative for EBNA-1 gene sequences were also negative for EBNA-2 gene sequences (Fig 1). There was a higher prevalence of EBV genome in those patients with nodal disease at diagnosis (6 of 9, 66%) compared with those who had extranodal disease at presentation (4 of 11; 36%; \( P = .37 \), two-tailed Fisher’s exact test). Four of the six patients with EBV-related nodal disease had B-type EBV in the tumor DNA (67%), whereas three of the four patients with EBV-related extranodal disease had A-type EBV (75%; \( P = .52 \), two-tailed Fisher’s exact test). Although this trend was not significant, it is possible some tropism exists among EBV subtypes for given sites.

There was also a trend for EBV tumor incorporation in the less immunodeficient patients. Eight of the 14 patients with CDC group II or III disease at diagnosis had EBV genome in their tumor DNA (57%), whereas only two of the six patients with a prior acquired immunodeficiency syndrome (AIDS) diagnosis had EBV in their tumors (33%; \( P = .62 \), two-tailed Fisher’s exact test). This trend was also evident when CD4 cell count was used to assess immunodeficiency, as 5 of the 8 patients with T4 counts over 200 × 10^6 cells/L at diagnosis (63%) had tumor EBV, whereas 4 of the 10 patients with counts less than 200 × 10^6 cells/L at diagnosis had EBV detectable in their tumors (\( N = 10; 40%; \ P = .64 \), two-tailed Fisher’s exact test). There was no correlation between histology and the presence of EBV genomes, with three patients having SNCC histology (\( N = 7; 43\% \) and six LCI histology (\( N = 12; 50\% \)). One patient’s tumor, high-grade by cytology criteria, could not be classified due to the small size of the biopsy.

There was no significant survival difference between those patients with EBV gene sequences in tumor tissue DNA and those without such sequences, nor was there a survival advantage for one EBV subtype over another.

Serology for EBV and HTLV-I

Nineteen patients were tested for antibodies to EBV nuclear antigens 1, 2A, and 2B (Table 2). The sera of all 10 patients with EBV in their tumor tissue contained antibodies to EBV antigens. Many of these sera showed dual reactivity to EBNA-2A and -2B antigens. EBV serology did not reliably predict the subtype of EBV in the tumor, nor was it helpful in predicting which patients had EBV-related tumors, as almost all subjects showed some reactivity to EBV antigens.

The HTLV-I serology was negative by both ELISAs in all 20 patients. Therefore, this retrovirus is unlikely to contribute to the pathogenesis of HIV-associated NHL.
Fig 2. PCR for the EBNA-1 gene. Samples 3 and 17 were amplified for EBNA-1 sequences starting with the following amounts of DNA (ng): tracks A, 500; tracks B, 50; tracks C, 5; tracks D, 0.5. Samples 2, 4, and 19 were amplified using 500 ng DNA. Molecular weight markers (bp) are shown on the left.

DISCUSSION

Ten of the 20 NHL patients in this study had EBV DNA in their tumor samples. This is consistent with previously published work using both Southern analyses and in situ techniques to assess the incidence of EBV in NHL tumors. Five of the patients in the present study had type B EBV and five had type A virus. Evidence for the presence of HIV or HTLV-I in these tumors was not obtained, confirming previous reports.

We were constrained by two difficulties in assembling the present collection of patient samples. Firstly, to obtain a reasonable number of samples for analysis, it was necessary to use formalin-fixed specimens, rather than awaiting fresh material from new cases. Secondly, in a number of cases, the available biopsy was very small. We chose to use PCR to analyze these samples because it is effective on formalin-fixed tissue and it requires only small amounts of tissue for analysis. A significant limitation with the PCR is that, because of its high sensitivity, it is possible that the positive signals for EBV and HIV DNA reported in this work may have arisen from small numbers of nontumor cells present in the biopsy. However, this is unlikely on a number of grounds. The overall frequency of EBV-positive tumors in this series is within the range published by others using different techniques. Furthermore, 90% of the patients had positive EBV serology (Table 2) whereas only 50% of the tumors were positive for EBV sequences (Fig 1). Therefore, we found 40% of the tumors did not contain EBV sequences, although they arose in patients with positive serology for EBV and in whom nontumor cells containing EBV DNA might have caused us to classify the tumors as positive for EBV. Indeed, three samples showed weak but consistently detectable EBNA-1 sequences (patients 10, 14, and 19; Fig 1). These sequences were only weakly present compared with the positive controls (Fig 2), a finding consistent with the notion that they were present in a minority of cells. Further evidence that the EBV signals arose from tumor cells was that in each EBV-positive tumor sample, only one EBNA-2 subtype was detected, although in 7 of these 10 cases both subtypes were detected serologically.

Initial studies of B-type EBV had suggested its distribution was limited to Central Africa and New Guinea. Later, B-type virus was found to be shed frequently from the nasopharynx of both HIV-infected and normal subjects, but peripheral blood B cells of normal subjects from North America who secreted both types of EBV showed only A-type virus incorporation. Work in progress suggests that B-type EBV is found in the peripheral blood of HIV-infected individuals. (Kyaw MT, Harren L, Evans L, Moss DJ, Cooper DA, Benson E, Esmore D, Sculley TB: in
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1

2

3

4

5

6

Fig 3. PCR for HIV sequences. Dilutions of DNA from the HIV infected cell line, Hut PW, were subjected to PCR, starting with the following amounts of DNA: track 2, 50 pg; track 3, 500 pg; track 4, 5 ng; track 5, 50 ng; track 6, 500 ng. Track 1 is from 500 ng DNA from sample 12. Molecular weight markers (bp) are shown on the left.

preparation). The studies reported here show that B-type EBV is present in nodal and extranodal sites in HIV-infected persons. Thus, it appears that B-type EBV has a much wider geographic distribution than originally thought and may be an important pathogen in HIV-infected subjects.

The serologic findings reported show that dual infection with both EBV subtypes is common in HIV-infected persons, as 11 of the 20 patients (55%) had specific antibodies to B-type virus and 10 (50%) had specific antibodies to both type A and B virus. Recent studies by another group showed dual secretion of EBV subtypes in throat washings from 50% of EBV secreting HIV-infected patients, again suggesting that many HIV-infected patients harbor both viral types.16 HIV-infected subjects are known to be defective in their ability to regulate EBV-infected B cells,11 and the HIV-induced deficiency in cell-mediated immunity in these patients may cause type B EBV spread beyond the nasopharynx. The equal frequency of A- and B-type EBV incorporation in lymphoid tumors from HIV-infected patients may be a reflection of the similar levels of infection of these two virus types in these subjects.

The high prevalence of type B EBV found in HIV-associated NHL is similar to the findings in endemic Burkitt's lymphoma, in which 40% of the tumors have type B viral sequences.15 This contrasts with the pattern of the c-myc translocation, found in 75% of HIV-associated NHL, that is typical of sporadic rather than endemic Burkitt's lymphoma.17,18,32,33 Three cases of phenotypically indeterminate HIV-associated NHL, which on molecular analysis proved to be EBV-positive, monoclonal B-cell tumors with germline c-myc on restriction enzyme analysis, have also been described.15 Thus, HIV-associated NHL has both similarities and differences with endemic Burkitt's lymphoma. The pattern of EBV subtyping and the relatively high frequency of EBV incorporation in tumor DNA is reminiscent of endemic Burkitt's lymphoma, but the c-myc changes are more typical of the sporadic form. Also, c-myc rearrangement and EBV incorporation in tumor DNA is found in all HIV-associated lymphoma histologies, not limited to the Burkitt-like, small noncleaved cell tumors.17,18,34-36

There are also distinctions between the lymphoproliferations of HIV-infected patients and those found in patients with other forms of immunodeficiency. Small, noncleaved cell histology is not a regular feature of posttransplant or chemotherapy-induced NHL.6 We and others have also found, using various techniques, that EBV is not associated with the lymphadenopathy syndrome of HIV infection77,79 (Boyle MJ, Sculley TB, Cooper DA, Turner JJ, Penny R, Sewell WA; in preparation), though it is a common finding in the benign and malignant lymphoproliferations of transplant recipients, in whom antiviral therapy is sometimes effective in controlling disease.80,81 The nature of the immunodeficiency is different in the various disease states and this will have an impact on the regulation of EBV and other infective agents, as well as a potential impact on the translocation of c-myc and any subsequent NHL. Indeed, endemic Burkitt's lymphoma is thought to arise in the setting of an immune deficiency consequent to malarial}

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infection. It may be that the explanation for these variations in c-myc translocations and in tumor histologies lies in the subtle differences in immune deficit in different patient groups, rather than being linked to the presence or absence of EBV in the tumor. Further studies will be needed to address this question.

EBV sequences tended to be more prevalent in the less immunodeficient patients, as assessed both by CDC group and CD4 cell count before the diagnosis of NHL, though the result did not reach statistical significance in the small number of patients studied. B-cell proliferation and tumor development may be more dependent on EBV-driven mechanisms in less immunodeficient patients. As immunodeficiency progresses, other mechanisms of B-cell proliferation and tumor induction may become more potent. Such alternative mechanisms may involve other viral cofactors, chronic antigenic stimulation, or drug-induced changes to the cells, as a number of the drugs used in patients with HIV infection have tumor-inducing potential. Further studies will be needed to address these questions. These studies suggest that EBV is an important cofactor in the development of NHL in HIV-infected persons and confirm previous reports that show no direct involvement of HIV or HTLV-I in these tumors. B-type EBV has previously been reported in oral hairy leukoplakia, and the finding that this virus is present in 25% of HIV-associated NHL supports the thesis that this type of EBV is an important human pathogen with a worldwide distribution.

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Subtypes of Epstein-Barr virus in human immunodeficiency virus-associated non-Hodgkin lymphoma

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