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Detection of Epstein–Barr Virus Genome in Ki-1 (CD30)-positive, Large-cell Anaplastic Lymphomas Using the Polymerase Chain Reaction

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Ki-1 (CD30)-positive, large-cell anaplastic lymphoma (LCAL) is a distinctive subset of non-Hodgkin's lymphoma; morphologically, the neoplastic cells of LCAL may closely resemble Reed–Sternberg cell variants of Hodgkin's disease. The neoplastic cells in Hodgkin's disease are often CD30-positive, as are some of the transformed lymphocytes in infectious mononucleosis. Recent evidence suggests an etiologic role for the Epstein–Barr virus (EBV) in Hodgkin's disease. Because of the phenotypic similarities between Hodgkin's disease and LCAL, we used the polymerase chain reaction (PCR) to analyze eight specimens of LCAL for EBV genome. Diagnoses were established by paraffin section morphology and immunohistochemistry. For comparison, we also analyzed nine non-Hodgkin's lymphomas other than the LCAL type, three Hodgkin's disease specimens, and nine non-neoplastic lymph nodes. PCR was performed using DNA extracted from frozen tissue; DNA was amplified using two sets of oligonucleotide primers corresponding to the BamH1 W-fragment of the EBV genome. Amplified EBV genome was obtained from all specimens except for one mantle zone lymphoma, one diffuse mixed-cell lymphoma, and six non-neoplastic lymph nodes. EBV terminus region probing and in situ hybridization techniques, each less sensitive than PCR, were performed in selected cases in an attempt to corroborate our PCR results. Only 2 of 13 specimens contained EBV detectable by these other techniques, and neither specimen was a LCAL. In view of the high incidence of latent EBV infections in humans, the biologic significance of our PCR results is uncertain. Despite the detection of EBV genome by PCR in a high percentage of lymphomas, we were unable to substantiate an etiologic role for EBV in LCAL. The PCR technique may be too sensitive to provide meaningful data on the possible role of EBV in lymphomagenesis. (Am J Pathol 1992, 141:457–465)

The Epstein–Barr virus (EBV) is recognized to be the etiologic agent of infectious mononucleosis.[1] Furthermore, multiple lines of evidence have revealed an association between EBV infection and endemic Burkitt's lymphoma,[2,3] post-transplant lymphoproliferative disorders,[4] some cases of Hodgkin's disease,[5–8] and some T-cell lymphomas.[9–11] In Hodgkin's disease, for example, epidemiologic, serologic, and molecular biologic analyses have supported an etiologic role for EBV in some cases. Molecular techniques applicable to such studies include genomic Southern blot hybridization (EBV terminus region probe), *in situ* hybridization, and the polymerase chain reaction (PCR). Several studies have demonstrated single clonotypic forms of the EBV genome in some cases of Hodgkin's disease,[7,8,12] supporting the contention that the viral infection occurred before neoplastic proliferation.[13]

Ki-1 (CD30)-positive, large cell anaplastic lymphoma (LCAL) is a distinctive subset of non-Hodgkin's lymphoma;[14–17] the neoplastic cells have morphologic and immunophenotypic (CD30-positive) similarities to Reed–Sternberg cells.[18–21] Furthermore, a recent report[22] has described atypical, CD30-positive immunoblasts in a lymph node from a patient with acute infectious mononucleosis. Because of these interrelationships, and because of the association of EBV with Hodgkin's disease,

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we undertook this study to look for evidence of the EBV genome in eight cases of LCAL.

Materials and Methods

Tissues

PCR analysis was performed on 19 frozen tissue specimens from 17 patients registered in the Southwest Oncology Group (SWOG) lymphoma repository. Diagnoses were established by SWOG reviewers using paraffin section morphology, frozen section immunophenotyping, and, in some cases, gene rearrangement analysis. Thirteen of the specimens were Ki-1 positive. Diagnoses included large-cell anaplastic lymphoma (eight specimens); other high-grade non-Hodgkin's lymphomas (two specimens); intermediate-grade non-Hodgkin's lymphoma (three specimens); low-grade non-Hodgkin's lymphoma (one specimen); Hodgkin's disease (three specimens); and reactive lymphoid hyperplasia (two specimens). Genomic DNA extracted from seven of these specimens, including four LCAL, was available for EBV terminus region probing. Paraffin sections of formalin-fixed tissue from nine of these specimens, including four LCAL, were available for *in situ* hybridization probing for EBV genome.

Ten frozen lymph node biopsies from ten patients were available from the University of Michigan Hospitals for PCR analysis. Lymph node diagnoses were established by paraffin section morphology. Diagnoses included low-grade non-Hodgkin's lymphoma (three specimens); dermatopathic lymphadenitis (one specimen); sarcoidosis (one specimen); and reactive hyperplasia, not further specified (five specimens).

Polymerase Chain Reaction

DNA Extraction for PCR

Six frozen sections (each section 4 μm thick; average sample area 36 mm^2) were cut from snapfrozen tissue blocks from each SWOG case. This volume of tissue was closely comparable to that used in a previous similar study.[5] New blades were used for each block to avoid cross-contamination of specimens. Each section was placed on a clean glass slide and fixed for 10 minutes in cold (4°C) ethanol. The tissue was scraped from the slides into microcentrifuge tubes, using a new, sterile blade for each case. Tissue digestion, DNA extraction, and DNA precipitation were performed according to previously described methods.[23] The precipitated DNA was dissolved in 10 μl of Tris-EDTA (TE) buffer and stored at 4°C.

Frozen tissue from the University of Michigan biopsies was available as tissue blocks rather than frozen sections. Each frozen tissue block (approx. 64 mm^3) was minced in cold Tris-NaCl-EDTA buffer using a new, clean blade. Tissue digestion, DNA extraction, and DNA precipitation were performed according to previously described methods.[23] DNA precipitates were dissolved in 50–75 μl of TE buffer. Because of the larger amount of available tissue, the concentration of extracted DNA was measured by optical densitometry at 260 nm.

DNA Amplification and Analysis

Oligonucleotide primers (Figure 1) corresponded to sequences in the internal repeat fragment (*Bam*H1 W-fragment) of the EBV genome; this fragment is reiterated ten times per genome.[24] These primers and the internal oligonucleotide probe were selected because their utility and specificity has been demonstrated by other investigators[5]; also, the reiterated structure of the target sequence would theoretically enhance the sensitivity of detection. The polymerase chain reactions were carried out in a Coy thermal cycler or a Perkin-Elmer Cetus (Emeryville, CA) thermal cycler at a reaction volume of 50 μl. All specimens were subjected to two rounds of PCR amplification, with 40 cycles in each round. Reaction mixtures for the first round of amplification contained: 10 mmol/l Tris-HCl, pH 8.3; 50 mmol/l KCl; 1.5 mmol/l $MgCl_2$; 0.01% gelatin; 2 mmol/l dithiothreitol; 0.2 mmol/l each of dATP, dCTP, dGTP, and dTTP; 1 μg of each

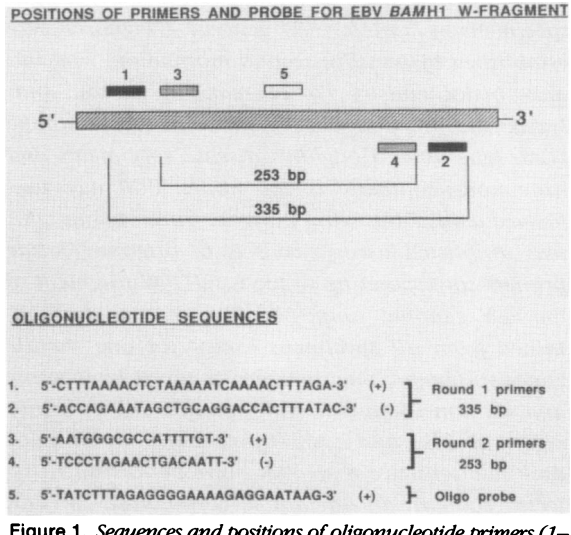

Figure 1. *Sequences and positions of oligonucleotide primers (1–4) and oligonucleotide probe (5) for* Bam*H1 W-fragment of EBV. (+) symbol represents "sense" strand of DNA; (−) symbol represents "anti-sense" strand of DNA.*

oligonucleotide primer; and template DNA from frozen section extracts (5 μl) or frozen tissue block extracts (approx. 100 ng). Based on unpublished observations from our laboratory, template DNA quantities in this range (50 ng–1.0 μg) yielded consistently efficient amplifications from known EBV-infected lymph node tissue.

The PCR reaction mixtures were heated to 94°C for 5 minutes and then cooled on ice for 2 minutes before adding 2.5 units of *Taq* polymerase. Each reaction mixture was covered with two drops of mineral oil and subjected to 40 cycles of amplification (each cycle = 1 minute at 94°C, 30 seconds at 55°C, and 1 minute at 72°C; final extension at 72°C for 10 minutes).

The first round of PCR was performed using oligonucleotide primers 1 and 2 (Figures 1, 2). A second round of 40 cycles was performed using nested primers 3 and 4 (Figures 1, 3). Template DNA for the second amplification round consisted of 4 μl of reaction product from the first round. All other reagents for the second round of amplification were in the same concentrations used for the first round. Positive control material consisted of DNA extracted from an EBV-infected RPMI leukemia cell line or from an EBV-infected lymph node from a patient with a transplant-associated lymphoproliferative disorder. Negative controls contained sterile water in place of template DNA. All specimens and controls were run in duplicate.

PCR products were electrophoresed on 2% agarose gels containing ethidium bromide (Figures 2, 3). These were examined and photographed by ultraviolet illumination. The gels were then transferred to nylon membranes and probed with ^{32}P end-labeled oligonucleotide 5 (Figures 1, 3b). Kodak X-OMAT AR film was exposed to the labeled blots for 1 hour to 5 days at −70°C using intensifying screens.

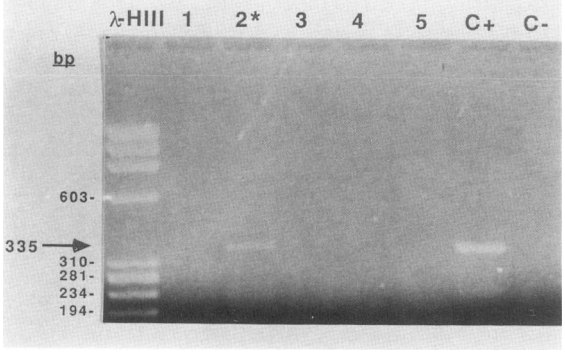

Figure 2. *Detection of EBV DNA by PCR after first round of amplification. 335-bp amplification product seen in lanes 2* and C+ (positive control) of ethidium bromide-stained gel. No bands visualized in lanes 1, 3, 4, 5 or C- (negative reagent control). λ-HIII is a size marker.*

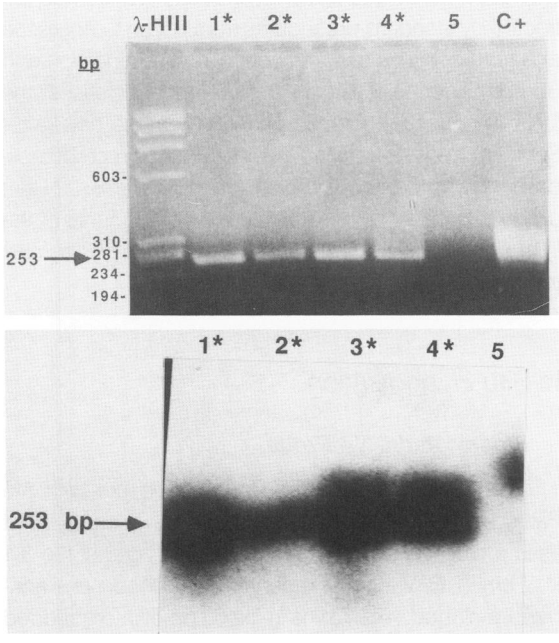

Figure 3. a: *Detection of EBV DNA by PCR after second round of amplification (not the same cases illustrated in Figure 2). 253-bp amplification product seen in lanes 1*–4* and C+ (positive control) of ethidium bromide-stained gel. No band visualized in lane 5.* **b:** *Southern hybridization of blot from gel shown in* **(a).** *Radioactively labeled oligonucleotide probe (sequence shown in Figure 1) has bound to 253-bp amplification product in lanes 1*–4*.*

Raji Cell Dilutions for Semiquantitative Assessment of PCR Sensitivity

To obtain a semiquantitative estimate of the sensitivity of our PCR method, we performed a dilutional study mixing varying numbers of Raji cells with an EBV-negative human leukemia cell line (RPMI 8402, T-cell leukemia line).[25] The Raji cell line is derived from a Burkitt's lymphoma and contains 50–100 copies of the EBV genome per cell.[26] Serial ten-fold dilutions of Raji cells, ranging from 10^6 to 10^1 cells, were mixed with RPMI 8402 cells to yield the combined equivalent of 2×10^6 human cellular genomes per tube.

Cellular digestion, DNA extraction, and PCR amplification were carried out as described for frozen tissue blocks. PCR product analysis was also performed as described earlier, using ethidium bromide-stained gels and Southern blot probing after each of the two rounds of amplification.

Southern Blot Analysis for EBV Terminus Region

To assess the presence and clonality of EBV genomes in the neoplastic tissues, an EBV terminus fragment probe

was used for Southern blot hybridization. DNA was extracted by standard procedures from frozen tumor tissue and from cell lines used as controls. After digestion with *Bam*H1, *Eco*R1, or *Bam*H1/*Hind*III endonucleases, the DNA was electrophoresed on 0.8% agarose gels. After Southern transfer to nylon or nitrocellulose membranes, probing was carried out with the EBV *Bam*H1 NJhet probe that detects the terminal repeat fragments of the EBV genome.[4] The probe was labeled with ^{32}P by nick translation.[27]

In Situ *Hybridization*

Biotinylated DNA Probe

The *in situ* hybridization was carried out with the Pathogene EBV kit (Enzo Diagnostics, New York). This kit uses a biotinylated DNA probe directed against the 3.1 kb *Bam*H1 EBV internal repeat region. Specimens analyzed included nine formalin-fixed, paraffin-embedded specimens from the SWOG lymphoma repository; formalin-fixed, paraffin sections of a case of EBV-infected, transplant-associated lymphoproliferative disorder (DNA from this case was used as a positive control for PCR); a formalin-fixed, paraffin-embedded cell block of Raji cells (cell culture line with 50–100 copies of EBV genome per cell); acetone-fixed cytocentrifuge preparations of Raji cells; a positive control (B95.8 marmoset cells) and a negative control (Ramos human lymphoblastoid cells) included with the Enzo kit.

Formalin-fixed, paraffin-embedded tissue sections were cut at 4 μm onto glass slides pretreated with 3-aminopropyltriethoxysilane. Slides were baked at 60°C for at least 1 hour. Specimen preparation, probe hybridization, and detection steps were carried out as described in the Enzo Pathogene kit instructions, with minor modifications. Endogenous peroxidase activity was inhibited by immersing the slides in a 1:1 mixture of 3% H_2O_2 and methanol. Tissue sections were digested with proteinase K (0.5 mg/ml in PBS-EDTA) for 10 minutes at 37°C. DNA denaturation was carried out in a convection oven at 100°C for 10 minutes. Probe hybridization was allowed to proceed overnight at 37°C. The avidin-biotin-peroxidase detection reagent was applied for 30 minutes at 22°C.

Radiolabeled DNA Probe

To enhance the sensitivity of EBV detection, *in situ* hybridization studies were also performed using a radioactively labeled probing method described previously.[28] This technique was applied to the nine formalin-fixed, paraffin-embedded specimens from the SWOG repository and to an EBV-infected, transplant-associated lymphoproliferative disorder. The DNA probe corresponded to the *Bam*H1 W-fragment of EBV, radiolabeled with ^{35}S by the random hexamer priming technique.[29] A known EBV-positive nasopharyngeal lymphoepithelioma was used as a positive control and known EBV-negative lymphoid tissue was used as a negative control.

Results

Polymerase Chain Reaction

Patient Specimens

Results of PCR analyses of the patient specimens are summarized in Table 1. After one round of PCR (including radioactive oligonucleotide probing), we detected EBV genomes in four of eight (50%) LCAL; five of nine (56%) non-Hodgkin's lymphomas other than LCAL; one of three (33%) Hodgkin's disease specimens; and three of nine (33%) non-neoplastic lymph nodes (Table 1, Figure 2).

After two rounds of PCR (including radioactive oligonucleotide probing), we detected EBV genomes in eight of eight (100%) LCAL; seven of nine (78%) non-Hodgkin's lymphomas other than LCAL; three of three (100%) Hodgkin's disease specimens; and three of nine (33%) non-neoplastic lymph nodes (Table 1, Figure 3).

Table 1. *Summary of Diagnoses and EBV PCR Results (n = 29)*

Diagnosis	No. of specimens	PCR round 1		PCR round 2	
		+ Et-Br	+ ^{32}P-probe	+ Et-Br	+ ^{32}P-probe
LCAL	8	0	4	8	8
NHL, high grade	2	0	1	1	2
NHL, int. grade	3	2	2	2	2
NHL, low grade	4	0	2	1	3
HD	3	1	1	2	3
Non-neoplastic	9	0	3	3	3
Total	29	3	13	17	21

Et-Br = ethidium bromide-stained gel; LCAL = large cell anaplastic lymphoma; NHL = non-Hodgkin's lymphoma; HD = Hodgkin's disease; int. grade = intermediate grade.

As noted previously, 13 of 19 SWOG specimens were positive for the CD30 (Ki-1) antigen; 12 of 13 Ki-1-positive specimens, and 4 of 6 Ki-1-negative specimens contained EBV genome detectable by PCR.

Raji Cell Dilution Experiment for Estimation of PCR Sensitivity

After the first round of PCR, as few as 10^3 Raji cells (diluted in 2×10^6 human cells) produced a distinct band at 335 bp on the ethidium bromide gel. After Southern blot probing, a band corresponding to as few as 10^2 Raji cells could be seen. Each Raji cell contains 50–100 copies of the EBV genome.[26] Therefore, in terms of viral genomes, one round of PCR can detect as few as 5×10^4 copies by ethidium bromide gel or 5×10^3 copies by Southern blot probing.

After the second round of PCR, as few as 10^2 Raji cells produced a distinct band at 253 bp on the ethidium bromide gel. This corresponds to approximately 5×10^3 viral genomes in 2×10^6 human cellular genomes. After Southern blot probing, a band corresponding to 10^1 Raji cells (or 5×10^2 viral genomes) could be seen.

Southern Blot Analysis for EBV Terminus Region

The seven specimens available for EBV terminus fragment probing included four LCAL, two Hodgkin's disease specimens, and one diffuse mixed-cell lymphoma. PCR had detected EBV genomes in all of these specimens, and all of them were CD30-positive. Only one of the specimens probed positive, i. e., was clonal for the EBV terminus region; this was a Ki-1–positive, diffuse mixed-cell lymphoma of T-cell lineage.

In Situ *Hybridization*

Biotinylated DNA Probe

The nine SWOG specimens available for *in situ* analysis included four LCAL, one large-cell immunoblastic lymphoma, one follicular small cleaved-cell lymphoma, one case of Hodgkin's disease, and two reactive hyperplasias. PCR had detected EBV genomes in all of these specimens except for the two lymph nodes with reactive hyperplasia. *In situ* hybridization analysis for EBV genomes was negative in all specimens tested.

Positive controls for *in situ* hybridization included the B95.8 cell line, a Raji cell block preparation, and a Raji cell cytocentrifuge preparation; all of these showed granular nuclear staining indicative of EBV DNA. The Ramos cell line (EBV-negative) and paraffin sections from a transplant-associated lymphoproliferative disorder (EBV-positive by PCR) were negative by *in situ* hybridization using the biotinylated EBV probe.

Radiolabeled DNA Probe

All but two of the specimens analyzed with the radiolabeled *in situ* probe yielded the same results as the biotinylated probe kit. The two discrepant specimens were an immunoblastic lymphoma (Figure 4) from the SWOG repository and a transplant-associated lymphoproliferative disorder (used as a positive control for PCR). These

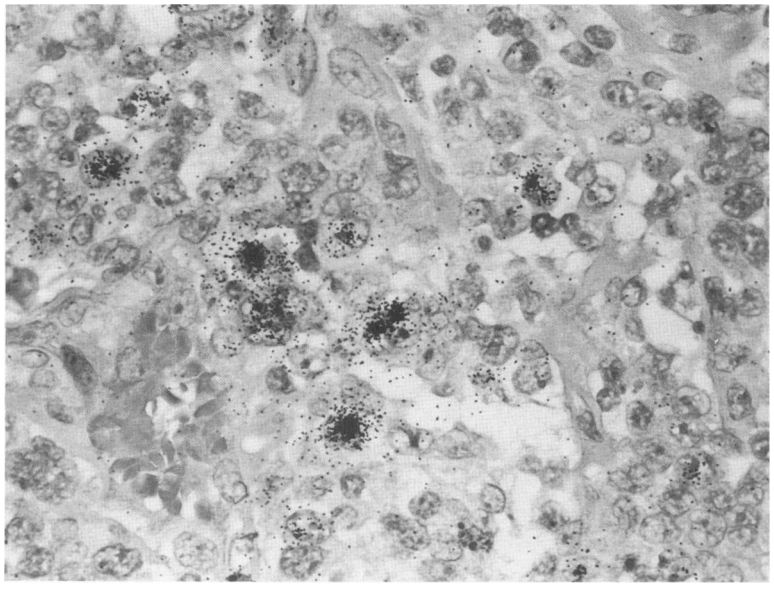

Figure 4. In situ *hybridization for EBV in a Ki-1–negative, immunoblastic lymphoma. Focal intense positivity noted in some immunoblasts. Strongly positive hybridization signal was restricted to an area of the neoplasm immediately adjacent to a zone of necrosis (not shown).*

two specimens were positive with the ^{35}S-labeled probe but negative with the biotinylated probe.

The immunoblastic lymphoma had a Ki-1-negative, B-cell phenotype. Using the ^{35}S-EBV probe, there was focal intense labeling of neoplastic cells adjacent to an area of necrosis (Figure 4); only rare cells away from this area were positive for EBV. In contrast to the focal pattern of hybridization observed in the immunoblastic lymphoma, the transplant-associated lymphoproliferative disorder showed a homogeneous pattern of positivity using the radiolabeled probe. A fairly strong hybridization signal was found in about 50% of the cells using the ^{35}S-labeled probe.

Discussion

By using PCR to amplify DNA extracted from 29 lymphoid tissue specimens, we detected EBV genomes in eight of eight (100%) large-cell anaplastic lymphomas; seven of nine (78%) non-Hodgkin's lymphomas other than LCAL; three of three (100%) Hodgkin's disease specimens; and three of nine (33%) non-neoplastic lymph nodes. Herbst and coworkers,[5] using a similar PCR protocol, reported a high incidence (58%) of EBV genomes in Hodgkin's disease; in addition, they found EBV genomes in 26% of high-grade non-Hodgkin's lymphomas and in 36% of hyperplastic lymph nodes analyzed by PCR.

Technique-dependent variability in PCR efficiency makes it difficult to compare the results of different studies in the literature. Quantitative estimates of PCR sensitivity are crude, and they are usually based on dilutional experiments[5,30] such as the one we performed using Raji cells. Herbst and colleagues[5] diluted EBV plasmid DNA in EBV-negative human DNA equivalent to 1–5 $\times$ 10^5 cellular genomes. They reported that the equivalent of 10^3 viral genomes reliably produced a band on ethidium bromide-stained gels after one round of PCR (30 cycles). This is roughly comparable to our sensitivity estimate for one round of PCR (40 cycles), detecting about 5 $\times$ 10^4 EBV genomes diluted in 2 $\times$ 10^6 EBV-negative human genomes.

Southern blot probing for PCR product and the use of nested amplification primers are maneuvers that can enhance the sensitivity of the PCR technique. For instance, in our Raji cell dilution experiment, we were able to increase the sensitivity approximately ten-fold by performing another round (40 cycles) of amplification using nested primers. A further ten-fold increase in sensitivity was achieved by the use of a radioactively labeled oligonucleotide probe for detection of PCR product.

Thus, the PCR method can achieve different levels of sensitivity depending on the amplification conditions and the method for detecting amplified product. Our results from the patient specimens would have been different if we had limited PCR amplification to one set of primers (one round of amplification) or had only used ethidium bromide for visualization of the amplified DNA. As shown in Table 1, one round of PCR amplification and ethidium-bromide staining yielded identifiable EBV product from only three of 29 specimens; two of the three were diffuse mixed-cell lymphomas and one was a case of Hodgkin's disease. A second round of amplification and oligonucleotide probing led to much greater sensitivity of detection, with positive results seen in 21 of 29 cases.

These results clearly demonstrate that the number of positive PCR cases was dependent on the laboratory method of detection, reflecting different levels of sensitivity. As such, one must be careful in interpreting published PCR studies and realize that varying conclusions might be drawn depending on the sensitivity of the PCR protocols that were used. This is especially true in studying viruses such as EBV, which are commonly found in the general population. Such studies should investigate a wide variety of cases to provide an appropriate frame of reference. By looking only at our LCAL cases by PCR, one might be tempted to surmise that EBV was playing an etiologic role in lymphomagenesis. By studying a spectrum of B- and T-cell non-Hodgkin's lymphomas, Hodgkin's disease, and benign lymph nodes with two rounds of PCR, however, we found that EBV can be detected in a variety of reactive and neoplastic lymphoid lesions.

Although these PCR data may suggest a possible association between EBV infection and some cases of Hodgkin's and non-Hodgkin's lymphomas, they are certainly insufficient by themselves to substantiate an etiologic role for the virus in such neoplasms. EBV is an ubiquitous agent with a high incidence of latent viral infection in the adult population. The exquisitely sensitive PCR technique may be detecting viral genomes from small numbers of latently infected, non-neoplastic lymphocytes infiltrating the neoplasms. Alternatively, it may be that PCR is detecting EBV genomes from lymphoma cells that have been infected after neoplastic transformation. In view of such interpretive difficulties, PCR data should be correlated with evidence gathered by other techniques.

Southern blot analysis of the genomic termini of EBV has been used to provide information regarding the clonality of EBV-associated neoplasms.[4,13] Specifically, the detection of a homogeneous population of EBV episomes in a neoplasm would indicate that the EBV infection preceded clonal expansion. Evidence of infection before clonal expansion would lend support to the postulate that the virus plays an etiologic role in tumorigenesis. For instance, Anagnostopoulos and colleagues[12] re-

ported a homogeneous population of EBV episomes in one of 22 CD30-positive LCAL that were studied.

We performed EBV terminus region probing in seven PCR-positive cases, including four LCAL, two Hodgkin's disease cases, and one diffuse mixed-cell lymphoma. In six cases, no EBV genome could be detected; this reflects the lower sensitivity of the Southern genomic technique compared with PCR. The only positive case was a Ki-1–positive, diffuse mixed lymphoma of T-cell phenotype. This neoplasm contained a homogeneous episomal EBV population, indicating that viral infection preceded clonal expansion. Not surprisingly, DNA from this case yielded a strongly positive signal for EBV after only one round of PCR amplification.

In situ hybridization is another useful technique because it may provide morphologic identification of the EBV-infected cells.[28] Like genomic probing for genomic termini, however, *in situ* analysis is much less sensitive than PCR. We applied the *in situ* technique to paraffin sections from nine SWOG specimens to localize the EBV genomes; EBV had been detected by PCR in all but two of these specimens. All nine specimens were negative for EBV using the biotinylated *in situ* probe. Only one SWOG specimen was positive using the more sensitive radiolabeled probe; this specimen was an immunoblastic lymphoma (Ki-1–negative) and it showed an unusual pattern of focal positivity near an area of tumor necrosis. The *in situ* pattern of focal EBV-positivity near an area of necrosis has been previously described in some cases of infectious mononucleosis lymphadenitis.[28,31] The significance of this finding in a case of immunoblastic lymphoma is uncertain. Unfortunately, no additional tissue was available for correlative EBV terminus region probing. Another puzzling feature of this case was the poor correlation between the *in situ* hybridization and PCR signals; *in situ* hybridization is much less sensitive than PCR, yet the PCR signal was not detectable until oligonucleotide probing was carried out after two rounds of amplification.

In conclusion, PCR amplified EBV genomes in all of the CD30 (Ki-1)–positive LCAL that we studied. In addition, EBV genomes were detectable in a large proportion of the other lymphomas analyzed by PCR, as well as in some hyperplastic lymph nodes. The biologic significance of these results is unclear. Certainly, investigators must be circumspect in drawing conclusions from PCR analyses, especially when studying ubiquitous viruses such as EBV. The exquisite sensitivity of PCR may actually confound issues from both a diagnostic and an experimental viewpoint. Without corroborative evidence from other molecular techniques, such as EBV terminus fragment probing or *in situ* hybridization, we cannot substantiate an etiologic role for EBV in the large-cell anaplastic lymphomas that we studied.

Similar concerns have been raised by other investigators studying the role of EBV in Hodgkin's disease. For example, Knecht et al[32] used PCR to identify EBV DNA in 38 of 48 (79%) Hodgkin's disease specimens. Included in their study were 17 cases with a high content (greater than 10%) of Reed–Sternberg or Hodgkin cells. In three cases with numerous neoplastic cells, no EBV DNA was found. In four other cases with numerous neoplastic cells, semiquantitative PCR showed only a few viral copies. These incongruities led the authors to suggest that EBV may have a modulating rather than a etiologic role in a substantial number of Hodgkin's disease cases.

The recent report of Masih et al[33] also casts doubt on the etiologic role for EBV in Hodgkin's disease. Using PCR, these investigators found EBV DNA in 58% of Hodgkin's disease cases and in 43% of hyperplastic lymph nodes. Cases were also studied by terminus fragment probing to detect EBV clonality. Of nine Hodgkin's disease cases from which episomal EBV DNA could be detected by Southern blot, the configuration was monoclonal in seven of them. Surprisingly, two of four hyperplastic lymph nodes with detectable episomal EBV DNA contained the viral genome in a monoclonal configuration. The authors suggest that detection of EBV genome in Reed–Sternberg cells may be a nonspecific phenomenon, not necessarily implying a pathogenetic role in the disease.

Defining the role of EBV in lymphomagenesis will require a better understanding of the molecular mechanisms of cellular transformation. Reports that show promise in this regard describe the immunohistochemical detection of EBV latent membrane protein restricted to the neoplastic cells of Hodgkin's disease.[34,35] The role of PCR in this area of research remains to be defined. Refinements in PCR quantitation may permit more accurate estimates of viral burden and help to define the relationship between viral burden and neoplasia. The detection of EBV clonality by PCR, if feasible, might also provide important information regarding EBV in the pathogenesis of lymphoma.

Acknowledgments

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