Detection of JC Virus DNA and Proteins in the Bone Marrow of HIV-Positive and HIV-Negative Patients: Implications for Viral Latency and Neurotropic Transformation

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Background.
We sought to determine the prevalence of JC virus (JCV) in bone marrow samples from human immunodeficiency virus (HIV)–positive and HIV-negative patients and to determine whether bone marrow is a site of latency and neurotropic transformation of JCV, the agent of progressive multifocal leukoencephalopathy (PML).

Methods.
We collected bone marrow aspirates, archival bone marrow samples, and blood and urine samples from 75 HIV-negative and 47 HIV-positive patients without PML as well as bone marrow and urine or kidney samples from 8 HIV-negative and 15 HIV-positive patients with PML. Samples were tested for JCV DNA by quantitative polymerase chain reaction and for JCV protein expression by immunohistochemical analysis. JCV regulatory regions (RRs) were characterized by sequencing.

Results.
JCV DNA was detected in bone marrow samples from 10 (13%) of 75 and 22 (47%) of 47 of the HIV-negative and HIV-positive patients without PML, respectively, compared with 3 (38%) of 8 and 4 (27%) of 15 of the HIV-negative and HIV-positive patients with PML. JCV DNA (range, 2–1081 copies/μg of cellular DNA) was detected in multiple leukocyte subpopulations of blood and bone marrow samples. JCV large T antigen, but not VP1 capsid protein, was expressed in bone marrow plasma cells. Bone marrow JCV RR sequences were similar to those usually found in the brains of patients with PML.

Conclusions.
Bone marrow is an important reservoir and a possible site of neurotropic transformation for JCV.

JC virus (JCV) [1] causes progressive multifocal leukoencephalopathy (PML), a deadly demyelinating disease of the central nervous system (CNS), in immunocompromised patients [2, 3]. Primary infection is asymptomatic and occurs during late childhood [4]. Approximately 86% of healthy adults are seropositive for JCV [5], and the virus remains quiescent in healthy individuals. In immunocompromised hosts, however, JCV can reactivate and cause a lytic infection of oligodendrocytes, resulting in PML.

The events leading to JCV reactivation and pathogenicity remain incompletely understood. Although the JCV coding region is very well conserved, its noncoding regulatory region (RR) is hypervariable and has been associated with neurovirulence. Indeed, a stable, nonpathogenic RR is usually found in intestinal samples from healthy and immunocompromised individuals, whereas the RR in brains of patients with PML is very well conserved. Although the JCV viral load is low in blood and cerebrospinal fluid of patients with PML, JCV can reactivate and cause a lytic infection of oligodendrocytes in the CNS.

Hematogenous spread is the likely mode of JCV dissemination into the CNS. Indeed, although healthy individuals usually do not carry JCV in their blood, several studies have shown that JCV DNA was detected in bone marrow aspirates from healthy and HIV-positive patients [6]. Bone marrow JCV RR sequences were similar to those usually found in the brains of patients with PML. Bone marrow JCV DNA was detected in bone marrow aspirates from 10 (13%) of 75 and 22 (47%) of 47 of the HIV-negative and HIV-positive patients without PML, respectively, compared with 3 (38%) of 8 and 4 (27%) of 15 of the HIV-negative and HIV-positive patients with PML.

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studies have shown JCV to be associated with peripheral blood leukocytes (PBLs) and cell-free plasma in immunosuppressed people [7–10]. However, the phenotype of cells carrying JCV in the bloodstream remains undetermined, because JCV has been found in association with B cells as well as other leukocyte subpopulations [11]. This question calls for further examination of the interactions between JCV and the hematopoietic system.

Little is known about the prevalence of JCV in the bone marrow of healthy individuals or immunocompromised patients. In 1988, Houff et al. [12] first demonstrated JCV DNA and capsid protein in bone marrow mononuclear cells from 2 patients with PML. Thereafter, JCV DNA detection has been reported in tonsils (21/54 [39%]) [13] as well as in the bone marrow of a few patients with PML [12, 14], patients with leukemia [15], and bone marrow transplant recipients [16]. More recently, the potential role of bone marrow as a reservoir of JCV has been highlighted by the occurrence of PML in 4 patients with multiple sclerosis (21/54 [39%]) [13] as well as in the bone marrow of a few patients with PML [12, 14], patients with leukemia [15], and bone marrow transplant recipients [16].

DNA extraction from fresh clinical samples. Sorted bone marrow and peripheral blood cell fractions were resuspended in 200 μL of PBS. DNA extraction was performed using the Qiagen DNA Blood Mini Kit. For urine, 200 μL of fluid was taken from the sample and used for direct extraction with the Qiagen MinElute Kit.

DNA extraction from archival bone marrow samples. Ten 5-μm-thick sections from the formalin-fixed, paraffin-embedded bone marrow blocks were collected into an Eppendorf tube, and a new microtome blade was used for each block. DNA was extracted after deparaffinization in 100% xylene for 10 min and then in 50% xylene and 50% ethanol for 10 min, followed by 100% ethanol for 10 min twice, all at 56°C. The dried sample was dissolved in 200 μL of tissue lysis solution, part of the Qiagen DNeasy blood and tissue kit. DNA was extracted according to the kit’s instructions.

Quantitative polymerase chain reaction (QPCR) for JCV. We used QPCR to detect and quantify JCV DNA in all samples, as described elsewhere [24]. The limits of detection for the assay were 100 copies/mL for JCV DNA from urine and 2 copies/μg for JCV PBL DNA.

Cloning and sequencing of JCV RR. We used the primers JC (5'-ATTCATTTCTCTCCCTGCATCATCTCTCTC-3'; nt 4846–4883) and JCR 422 (5'-TTTTTGGTCTACACTGTCCATCCCTG-3'; nt 425–398). For each PCR, 20 pmol of primers was used in 30 cycles of amplification with an annealing temperature of 63°C. The PCR products corresponding to the expected size were cloned with a TOPO TA Cloning Kit (Inviogen). Ten clones for each PCR product were analyzed by restriction enzyme digestion and electrophoresis. We sequenced 10 clones per each amplified fragment length. The DNA sequence was obtained on an ABI 3730xl sequencer (Applied Biosystems). Sequence analyses were performed using Lasergene MegAlign software (version 7.1; DNA STAR).
Table 1. Detection of JC virus DNA by quantitative polymerase chain reaction in fresh clinical samples.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>BM aspirates</th>
<th>PBLs</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>4/13</td>
<td>4/11</td>
<td>6/10</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>1/4</td>
<td>0/3</td>
<td>NA</td>
</tr>
<tr>
<td>Leukemia</td>
<td>0/4</td>
<td>0/2</td>
<td>1/1</td>
</tr>
<tr>
<td>MGUS</td>
<td>2/5</td>
<td>1/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Anemia</td>
<td>1/4</td>
<td>1/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Infections</td>
<td>1/2</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1/2</td>
<td>0/1</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>10/34 (29)</td>
<td>6/24 (25)</td>
<td>7/17 (41)</td>
</tr>
</tbody>
</table>

NOTE. Data are proportion (%) of positive samples. BM, bone marrow; MGUS, monoclonal gammopathy of unknown significance; NA, not available; PBLs, peripheral blood leukocytes.

Immunohistochemistry (IHC) staining. IHC staining was performed using the following antibodies: VP1 (PAB597; gift from W. Atwood), large T antigen (Tag; SV40 Tag [v-300]; Santa Cruz Biotechnology), and CD20, CD8, CD4, CD3, and CD138 (Dako), as described elsewhere [25]. Antigen retrieval was performed in boric acid (0.2 mol/L) at 60°C overnight.

Statistical analysis. Correlation between JCV DNA detection and CD4+ cell counts, receipt of highly active antiretroviral therapy (HAART), or HIV load was performed using Student’s t test (2-tailed). Comparison between JCV DNA detection in the bone marrow samples from HIV-positive and HIV-negative patients was performed using Fisher’s exact test (2-tailed).

RESULTS

Detection of JCV DNA in fresh bone marrow aspirates. We tested fresh bone marrow aspirates from 34 patients without PML (table 1). Using QPCR analysis, we found that 10 (29%) of 34 of these bone marrow aspirates had at least 1 cell subpopulation that was positive for JCV. Furthermore, 6 (25%) of 24 of the matching peripheral blood samples obtained at the time of bone marrow aspiration had at least 1 cell subpopulation containing JCV DNA. Finally, 7 (41%) of 17 of the urine samples from these patients had detectable JCV DNA. Every patient with detectable JCV DNA in PBLs also had detectable JCV in bone marrow. However, 4 patients with JCV-positive bone marrow aspirate did not have detectable JCV DNA in PBLs. The presence of JCV in urine was not correlated with detection in bone marrow and peripheral blood. Only 2 of the 7 positive urine samples were from patients with detectable JCV DNA in bone marrow or PBLs. Of these 34 patients, 6 (18%) were HIV positive. Three of these 6 patients (50%) had detectable JCV in bone marrow, compared with 7 of 28 (25%) among the HIV-negative patients. Two of these 3 HIV-positive patients with detectable JCV DNA in bone marrow also had detectable JCV in PBLs.

Of the 10 bone marrow aspirates with detectable JCV (table 2), the first 8 were sorted by flow cytometry, and samples 9 and 10 were sorted by means of an autoMACS cell sorter. When available, PBLs were also sorted by the same set of surface markers and using the same methods. JCV DNA was detected in multiple cell subpopulations, including PMN cells, monocytes, and B, T, NK, and NKT cells, as well as the unsorted fraction and the fraction remaining after sorting. JCV DNA detection was not associated with any particular subpopulation. Overall, the JCV loads in both bone marrow and PBL were low. The highest JCV load in bone marrow was 250 copies/μg of cellular DNA in NK cells from patient 3. The lowest JCV load was 2 copies/μg of cellular DNA in the unsorted samples from patients 2 and 5. The highest JCV load in peripheral blood was 1081 copies/μg in the remaining fraction from patient 6. Detection of JCV DNA in a given cell type from the bone marrow did not always correspond to its presence in the same cell type in peripheral blood from the same patient. Patient 5, who was HIV positive and had anemia, had 5 sorted cell subpopulations that contained JCV DNA.

JCV DNA detection in archival bone marrow samples from HIV-positive and HIV-negative patients. We sought to explore further the effect of HIV infection on JCV detection in bone marrow samples. We identified 88 patients (41 HIV-positive and 47 HIV-negative) with archival bone marrow samples, obtained at Beth Israel Deaconess Medical Center from 2003 through 2007. The indications for bone marrow biopsies in HIV-positive and HIV-negative patients.

To determine whether JCV was present in the bone marrow of patients with active JCV replication in their brains, we tested 8 HIV-negative and 15 HIV-positive patients with PML. Bone marrow samples from the 8 HIV-negative patients with PML (table 4), including 3 fresh bone marrow aspirates and 5 archival samples, were obtained 7–139 days after PML diagnosis. Of 8 bone marrow samples, 3 (38%) had detectable JCV DNA. We obtained matching
peripheral blood samples from 7 and urine samples from 5 of these HIV-negative patients with PML. Of the 3 patients with detectable JCV DNA in bone marrow, 2 also had detectable JCV DNA in PBLs, and only 1 had detectable JCV DNA in urine. In the 15 HIV-positive patients with PML, archival vertebral bone and kidney specimens obtained at autopsy were tested. Of those, 4 patients (27%) had detectable JCV DNA in bone specimens, and only 1 had detectable JCV DNA in the kidney. Four other patients had detectable JCV DNA in kidney specimens only.

Grouping all of our samples by HIV and PML status, we detected JCV DNA in the bone marrow samples from 10 (13%) of 75 HIV-negative and 22 (47%) of 47 HIV-positive patients without PML (P < .001), compared with 3 (38%) of 8 HIV-negative and 4 (27%) of 15 HIV-positive patients with PML (P = .65).

**JCV protein detection in archival bone marrow samples.** To determine whether JCV remained quiescent in bone marrow or underwent a productive cycle and expressed proteins, we then performed IHC staining on all archival samples that had detectable JCV DNA by QPCR. None of the samples had detectable JCV VP1 capsid protein. However, JCV large TAg was found in 6 (32%) of 19 HIV-positive samples and in 2 (67%) of 3 HIV-negative samples that had detectable JCV by QPCR, respectively. Samples that were negative by QPCR were used as controls for TAg staining. To characterize the specific cell type supporting JCV TAg expression, we performed double IHC staining with cell markers on those samples that were positive for TAg. These experiments showed that TAg was mostly present in cells that also stained for CD138, a plasma cell marker (figure 1).

**Characterization of JCV RR sequences in bone marrow.** To determine the type of the JCV RR present in archival bone marrow samples, we sequenced the RR after PCR amplification and cloning. Among archival samples that were positive for JCV

### Table 2. Quantification of JC virus (JCV) load by quantitative polymerase chain reaction in sorted cell subpopulations from bone marrow (BM) aspirates and peripheral blood leukocytes (PBLs).

<table>
<thead>
<tr>
<th>Patient (diagnosis), sample type</th>
<th>Cell population</th>
<th>Cell population</th>
<th>Cell population</th>
<th>Cell population</th>
<th>Cell population</th>
<th>Cell population</th>
<th>Cell population</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (MGUS)</td>
<td>BM</td>
<td>PBLs</td>
<td>BM</td>
<td>PBLs</td>
<td>BM</td>
<td>PBLs</td>
<td>BM</td>
</tr>
<tr>
<td>2 (MGUS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (infection)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (multiple myeloma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (anemia; HIV positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (thrombocytopenia; HIV positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (lymphoma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (lymphoma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (lymphoma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (lymphoma; HIV positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are JCV copies/μg. Minus signs indicate that JCV was not detected. MGUS, monoclonal gammopathy of unknown significance; Mono, monocytes; NA, not available; NK, natural killer; NKT, natural killer T; PMN, polymorphonuclear.
by QPCR, we were able to amplify 6 of 19 samples from HIV-positive patients and 1 of 3 from HIV-negative patients. All 7 RRs had tandem repeats of the 98-bp element, similar to the Mad-1 JCV prototype. However, each patient sample contained unique point mutations in either the first and/or second 98-bp repeat element or in the agnop gene. We were not able to obtain sequences from JCV RRs in fresh bone marrow aspirates or peripheral blood samples.

**DISCUSSION**

In our quest to better understand the mechanisms that lead to JCV pathogenesis and dissemination, our goals were to determine the prevalence of JCV in bone marrow and characterize the phenotype of JCV-infected cells. We therefore combined a highly specific cell sorting method with a very sensitive QPCR assay, which demonstrated that 29% of fresh bone marrow aspirates contained JCV DNA. The bone marrow is an ideal site for long-term viral latency, as seen with the herpesviruses, Epstein-Barr virus (EBV), and cytomegalovirus [26, 27]. Furthermore, the bone marrow contains progenitor and mature cells that are known to be susceptible to JCV infection. Indeed, JCV DNA has been found in B lymphocytes in peripheral blood [7]. In vitro, JCV can infect both primary CD34+ cells and primary tonsillar B lymphocytes [9]. Finally, nuclear protein extracted from B lymphocytes can bind to nuclear factor–1 sites in the JCV RR to promote JCV replication [10]. Our demonstrations of a high prevalence of JCV in bone marrow indicate that the bone marrow compartment harbors JCV. Moreover, because all subjects with JCV viremia also had JCV in bone marrow, the virus may spread throughout the body via the hematogenous route.

HIV infection is the major risk factor for PML, and ~80% of patients with PML have AIDS [28, 29]. Our data indicate that bone marrow JCV is significantly more prevalent in HIV-positive than in HIV-negative patients with similar hematological conditions. One possible explanation is that HIV-positive patients experience a prolonged and more-severe immunosuppression that may promote JCV reactivation. Others have shown evidence of molecular interactions, either direct or indirect, between HIV and JCV [30–34]. Because the presence of HIV in bone marrow has been demonstrated [35, 36], it is therefore possible that interplay between JCV and HIV in this compartment is an important factor in JCV neurotropic transformation.

In the bone marrow, JCV was detected in sorted cell groups, including PMN cells, monocytes, B cells, T cells, NK cells, and NKT cells, and in the cells that remained after sorting, which include progenitor cells before the expression of mature cell markers and hematological abnormal cells. This is consistent with our previous observation that JCV DNA can be associated with multiple leukocyte subpopulations in peripheral blood [11]. Interestingly, the sorted CD34+ cell group did not have any detectable JCV. However, our ability to detect JCV DNA in the CD34+ cell subpopulation may have been limited by the small percentage of CD34+ cells in human bone marrow [37]. The sorted CD34+ cell group always contained 10–100 times fewer cells than the other groups.

To determine whether bone marrow harbors quiescent JCV or sustains a productive infection by this virus, we performed IHC staining of archival bone marrow samples from HIV-positive and HIV-negative patients, which showed expression of JCV TAg in a third of those with detectable JCV DNA but no expression of the VP1 protein. TAg is a regulatory protein that is expressed early in the viral life cycle, is crucial for JCV DNA replication [38, 39], and has been associated with cellular oncogenic transformation [40]. Conversely, the VP1 protein is the major component of the viral capsid and is expressed at the time of viral assembly. These data suggest that JCV may remain quiescent in bone marrow or undergo an abortive, rather than a productive, infectious cycle.

Among patients without PML, JCV DNA was significantly more prevalent in the bone marrow of HIV-positive patients (22/47 [47%]) than in that of HIV-negative patients (10/75 [13%]) (P < .001). Interestingly, this difference was accentuated in the subset of HIV-positive and HIV-negative patients

Table 3. Detection of JC virus DNA by quantitative polymerase chain reaction in archival bone marrow (BM) samples from HIV-positive and HIV-negative patients.

<table>
<thead>
<tr>
<th>Indication for BM biopsy</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>2/6</td>
<td>1/8</td>
<td>NS</td>
</tr>
<tr>
<td>Spleomegaly</td>
<td>1/1</td>
<td>0/1</td>
<td>NS</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>0/2</td>
<td>0/2</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0/1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MGUS</td>
<td>0/2</td>
<td>0/2</td>
<td>NS</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>1/1</td>
<td>0/5</td>
<td>NS</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>5/8</td>
<td>1/7</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>10/20</td>
<td>1/22</td>
<td>.001</td>
</tr>
<tr>
<td>Total</td>
<td>19/41 (46)</td>
<td>3/47 (6)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

**NOTE.** Data are proportion (%) of positive samples. MGUS, monoclonal gammopathy of unknown significance. NA, not available; NS, not significant.

* Fisher’s exact test, 2-tailed.

Table 4. Detection of JC virus DNA by quantitative polymerase chain reaction in clinical samples from HIV-positive and HIV-negative patients with progressive multifocal leukoencephalopathy (PML).

<table>
<thead>
<tr>
<th>Patients with PML</th>
<th>BM</th>
<th>PBLs</th>
<th>Urine</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV negative</td>
<td>3/8</td>
<td>2/7</td>
<td>4/5</td>
<td>NA</td>
</tr>
<tr>
<td>HIV positive</td>
<td>4/15</td>
<td>NA</td>
<td>NA</td>
<td>5/11 (45)</td>
</tr>
<tr>
<td>Total</td>
<td>7/23 (30)</td>
<td>2/7 (29)</td>
<td>4/5 (80)</td>
<td>5/11 (45)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are proportion (%) of positive samples. BM, bone marrow; NA, not available; PBLs, peripheral blood leukocytes.
RR, which is present in most urine samples, the Mad-1–like RRs contaminate between patients samples. Unlike the archetype repeat, ruling out a contamination with Mad-1 plasmid and cross-contaminations between patients samples. Multiple clones from each specimen contained unique point mutations in either the first or second 98-bp repeat, ruling out a contamination with Mad-1 plasmid and cross-contaminations between patients samples. Unlike the archetype RR, which is present in most urine samples, the Mad-1–like RRs containing tandem repeats of a 98-bp element are usually detected in CNS samples from patients with PML [6] and also in the blood of patients with PML who have a poor outcome [50]. This finding suggests that the bone marrow may be the site of JCV neurotropic transformation. These results confirm our initial observation that archetypal and rearranged RRs with a tandem repeat pattern may coexist in the bone marrow of an HIV-negative patient with PML and rheumatoid arthritis [23].

There are several limitations to our study. First, because of the difficulties in collecting large numbers of bone marrow samples, our specimens are heterogeneous. Indeed, the efficiency of JCV DNA extraction and detection by PCR may vary in fresh bone marrow aspirates or formalin-fixed, paraffin-embedded biopsy samples. Therefore, we chose to process samples and analyze data separately according to the type of specimen obtained (tables 1 and 3). One exception involved bone marrow samples from patients with PML, which are extremely rare; only archival vertebral bone samples, rather than iliac crest biopsy samples, were available from HIV-positive patients with PML. Nevertheless, these results suggest that viral persistence in bone marrow may not be necessary at the time of active viral replication in the brain. Second, we may have underestimated the prevalence of JCV in fresh bone marrow aspirates. Indeed, of 34 samples, 25 were sorted, and the remaining 9 were extracted as a whole. Although 10 of the sorted samples contained detectable JCV DNA, none of the 9 unsorted samples were positive. Because of the low JCV load in bone marrow, the larger quantity of chromosomal DNA may have diluted the small amount of JCV DNA present in unsorted samples. Finally, we were able to clone and sequence JCV RR only from formalin-fixed, paraffin-embedded bone marrow biopsy samples, probably because of the low JCV load in fresh bone marrow aspirates and peripheral blood. This limited our ability to compare the JCV RRs in the bone marrow and peripheral blood from the same individuals, which could aid in clarifying JCV latency and reactivation.

In conclusion, this is the first study to show the prevalence of JCV in bone marrow from both HIV-positive and HIV-negative patients with or without PML. The presence of JCV DNA and TAg and the neurotropic RRs implicate bone marrow as being an important site in the pathogenesis of JCV infection. A greater understanding of primary JCV infection, latency, and reactivation is urgently needed to prevent the occurrence of PML in a growing number of patients treated with novel immunomodulatory therapies for cancer and autoimmune diseases.

Acknowledgments

We are grateful to Dr. Susan Morgello, Benjamin B. Gelman, H. Aaron Aronow, Elyse Singer, and Deborah Commins for providing PML samples through the National NeuroAIDS Tissue Consortium, which is supported by National Institutes of Health grants R24MH59724, R24NS38841, R24MH59745, and R24MH59656. We thank the following physicians for

with lymphoma (10/20 [50%] vs. 1/22 [5%]) (P = .001). The role that JCV might play in HIV-associated lymphomas deserves further study. Before the HAART era, HIV seropositivity increased the risk of lymphoma 60–165-fold [41–43], and this association has not changed markedly since the availability of HAART [44]. Hence, the pathogenesis of HIV-associated lymphomas is likely multifactorial [45], including the induction of cytokines resulting in B cell proliferation, prolonged immunosuppression, and inability to control oncogenic viruses, such as the herpesviruses EBV and human herpesvirus 8 [46, 47], which may in turn cause genetic alterations in B cells. A previous study showed that CNS lymphomas contained a significant quantity of JCV genome and that JCV TAg can be coexpressed with EBV in the same cells [48]. In this context, the presence of JCV TAg, an oncogenic protein, in bone marrow cells from the B cell lineage is a tantalizing finding.

Having ascertained the presence of JCV in bone marrow, we sought to characterize JCV RR in this compartment, because this noncoding region contains determinants of neurotropism and neurovirulence. However, the task was rendered difficult by the low JCV load found in bone marrow. All of our cloned JCV RRs from bone marrow samples were similar to the JCV Mad-1 prototype [49], but multiple clones from each specimen contained unique point mutations in either the first or second 98-bp repeat, ruling out a contamination with Mad-1 plasmid and cross-contaminations between patients samples. Unlike the archetype RR, which is present in most urine samples, the Mad-1–like RRs

Figure 1. Double immunostaining for JC virus (JCV) T antigen (TAg) and CD138+ plasma cells in an archival bone marrow sample from an HIV-positive patient without progressive multifocal leukoencephalopathy. Three CD138+ plasma cells (red) express JCV TAg (brown, arrows), next to 1 uninfected plasma cell (red only, asterisk) and 1 TAg+CD138+ cell (brown only, arrowhead). The bar represents 50 μm.

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