Posttransplantation Lymphoproliferative Disorders Frequently Contain Type A and Not Type B Epstein-Barr Virus

By Dale Frank, Ethel Cesarman, Yi Fang Liu, Robert E. Michler, and Daniel M. Knowles

Two families of Epstein-Barr virus (EBV), type A and type B, have been defined on the basis of sequence divergence in the EBNA-2 gene. Type A EBV immortalizes B cells more efficiently in vitro and infects immunocompetent individuals more commonly than type B EBV. However, increased rates of infection by type B EBV are seen in immunocompromised hosts and in many lymphoid neoplasms associated with immunocompromise. The posttransplantation lymphoproliferative disorders (PT-LPDs) are a heterogeneous group of B-cell neoplasms that arise in the setting of immunosuppressive therapy and are associated with EBV infection. Whether type A and/or type B EBV are associated with PT-LPDs is unknown. Therefore, we investigated 27 PT-LPD lesions from 22 solid-organ transplant recipients by polymerase chain reaction (PCR) at the EBNA-2 and EBNA-3c loci to detect sequence deletions that distinguish the two EBV families. Another focus, EBER, was examined by single-strand conformation polymorphism analysis (SSCP), in conjunction with direct sequencing in selected cases. Type A EBV was found in 24 of 27 cases (89%) as seen by amplification of the EBNA-2 and EBNA-3c regions. Four different EBER polymorphisms were detected, confirming the presence of different type A EBV isolates among these cases. Three cases were negative for infection by EBV. Surprisingly, despite the immunocompromised state of the hosts, none of the 27 PT-LPD lesions harbored type B EBV. Thus, although type B EBV may commonly infect peripheral blood lymphocytes in immunocompromised individuals, they do not appear to induce readily PT-LPD formation.

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GENOMIC ANALYSES of Epstein-Barr virus (EBV) isolates from around the world have identified two broad families of EBV, designated type A and type B, or 1 and 2, respectively. These were initially defined on the basis of divergence within the coding region for EBNA-2, a viral gene expressed during latent infection that is crucial for B-cell immortalization. The two families vary in their geographic distribution, tissue tropism, and biologic behavior. Type A EBV is ubiquitous, while type B EBV is commonly isolated only in parts of Africa endemic for malaria and Burkitt’s lymphoma. Type A EBV can infect oropharyngeal epithelial cells and peripheral blood lymphocytes, while type B EBV, when found in healthy Westerners, is seen in the oropharynx and only rarely in the peripheral blood. Type A EBV efficiently immortalizes B cells, while type B EBV immortalizes B cells far less effectively in vitro, and these B cells are less hardy in culture than those infected by type A EBV.

Many studies have shown an increased incidence of oropharyngeal and peripheral blood infection by type B EBV in various immunocompromised populations. A higher rate of type B infection, for example, is found in people living in equatorial Africa, and may be related to the generalized depression of type B infection, for example, is found in people living in equatorial Africa, and may be related to the generalized depression in T-cell function and intense polyclonal B-cell stimulation commonly seen in this part of the world. In the West, patients debilitated by age and illness have been found to harbor type B EBV. An increased incidence of infection by type B virus has been shown in people with acquired immunodeficiency syndrome (AIDS), in whom an EBV-specific depression in cell-mediated immunity has been demonstrated. Infection by type B EBV also appears to be more common in some lymphoid neoplasms associated with immunocompromise, such as AIDS-associated non-Hodgkin lymphomas, of which 50% of those that are positive for EBV have been shown to contain type B EBV.

In accordance with these findings, it has been shown that up to one third of cardiac transplant patients on chronic immunosuppressive therapy carry type B EBV in their peripheral blood, a much higher figure than the 3% observed in the general population. However, the relationship of type B EBV with the development of the posttransplantation lymphoproliferative disorders (PT-LPDs), a heterogeneous group of B-cell neoplasms that arise in the setting of immunosuppressive therapy and are associated with EBV infection, is unknown. We evaluated a panel of 27 PT-LPDs, ranging from polyclonal B-cell expansions to frank malignant lymphomas and multiple myelomas, to determine the type of EBV associated with these lesions.

MATERIALS AND METHODS

Pathologic samples. One lung, two kidney, and 19 heart transplant recipients who developed one or more lymphoproliferative disorders following organ transplantation at the Columbia Presbyterian Medical Center during the period April 1988 to February 1993 were included in this study. The clinical features of these patients and the pathologic and molecular genetic features of their PT-LPDs have been described elsewhere. Their inclusion was based on the availability of a minimum of one fresh, unfixed tissue specimen sufficient in quantity to permit the studies described below. Twenty-seven PT-LPD specimens were collected from the 22 patients during the course of clinical evaluation using standard diagnostic procedures as follows: lymph nodes, n = 9; lungs, n = 6; tonsils and/or adenoids, n = 5; jejunum, n = 1; colon, n = 1; liver, n = 1; brain, n = 1; breast, n = 1; bone marrow, n = 1; and soft tissues, n = 1. Twenty-six of 27 PT-LPD specimens were collected under sterile conditions, placed in tissue culture medium RPMI 1640 or physiologic saline, and immediately delivered to the Hematopathology Laboratory. Rep-
representative portions of these 26 specimens were snap-frozen in a cryopreservation solution (OCT compound; Miles, Elkhart, IN) and stored at −70°C. The remaining specimen was collected in a heparinized syringe. A viable mononuclear-cell suspension was prepared from this sample by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density-gradient centrifugation. The cells were cryopreserved in dimethylsulfoxide and fetal calf serum (FCS) in vapor-phase liquid nitrogen at −170°C. Portions of each of the 27 PT-LPD specimens were routinely fixed in formalin, B5, and/or Bouin’s, and embedded in paraffin from which hematoxylin-and-eosin–stained sections were prepared.

**Cell lines.** EBV-infected B-lymphoblastoid cell lines B95-8 and Stork, EBV-infected Burkitt’s lymphoma cell lines AG876 and EB-3, and promyelocytic leukemia cell line HL60 (not infected by EBV) were grown at 37°C in tissue culture medium RPM1 1640 containing 10% FCS in a humidified atmosphere containing 5% CO2.

**DNA extraction.** Genomic DNA was extracted from cryopreserved tissue blocks and mononuclear-cell suspensions using a salting-out procedure. Briefly, the cells or frozen-tissue sections were resuspended in 3 mL of nuclei lysis buffer containing 10 mmol/L Tris HCl, 400 mmol/L NaCl, and 2 mmol/L EDTA; 200 μL of 10% sodium dodecylsulfate, NaDodSO4 (SDS), and 500 μL of proteinase K solution (1 mg proteinase K in 1% SDS and 2 mmol/L EDTA) were subsequently added. After overnight digestion at 37°C, 1 mL of saturated NaCl was added. This mixture was centrifuged at 2,500 rpm for 20 minutes, and 2 vol of ethanol was added to the supernatant to precipitate the DNA, which was washed several times in 70% ethanol.

**Oligonucleotide primers and probes.** The oligonucleotides used in this study as primers for polymerase chain reaction (PCR) amplification and as probes for Southern blotting were synthesized by the solid-phase triester method. Primers and probes for the EBNA-2, EBNA-3c, and EBER regions were the same as those reported by reference controls, and the HL60 promyelocytic leukemia cell line was used as a negative control in our analyses of the PT-LPD region.

**PCR amplification.** PCRs were performed with 100 ng of genomic DNA, 10 pmol of each primer, 2.5 μmol/L dNTPs (for EBNA-2), 12.5 μmol/L dNTPs (for EBNA-3c), or 50 μmol/L (for EBER), 10 mmol/L Tris-HCl (pH 9.0), 50 mmol/L KCl, 0.1% Triton X-100, 1.5 mmol/L MgCl2 (for EBNA-2) or 1.0 mmol/L (for EBNA-3c and EBER), and 0.5 U Taq polymerase, in a final volume of 10 μL. Thirty cycles of denaturation (30 seconds at 94°C), annealing (1 minute at 58°C), and extension (2 minutes at 72°C) were performed on an automated heat-block (DNA Thermal-Cycler; Perkins-Elmer Cetus, Norwalk, CT). Reaction products were fractionated by electrophoresis in 3.0% agarose, and visualized under UV light by ethidium bromide fluorescence.

**Southern blot hybridization analysis.** PCR products were electrophoresed in 1.0% agarose gels, denatured with alkali, neutralized, and transferred to nitrocellulose filters according to Southern.27 Filters were prehybridized for 2 hours at 37°C and hybridized with a 3P end-labeled probe for 12 hours at 37°C in a solution consisting of 5X SSPE (1X SSPE = 150 mmol/L NaCl, 10 mmol/L NaH2PO4, H2O, 1 mmol/L EDTA), 5X Denhardt’s solution, 0.5% SDS, and 0.1 mg/mL salmon-sperm DNA. Filters were washed twice in a solution consisting of 2X SSPE and 0.1% SDS for 15 minutes at room temperature, once in a solution consisting of 5X SSPE and 0.1% SDS for 10 minutes at 54°C (EBNA-2) or 51°C (EBNA-3c), and rinsed briefly in 2X SSC (1X SSC = 150 mmol/L NaCl, 15 mmol/L Na3Citrate·2H2O). Autoradiography was performed at −70°C with an intensifying screen for 30 minutes to 2 hours.

**Single-strand conformation polymorphism analysis.** Single-strand conformation polymorphism (SSCP) analysis of the EBER region was accomplished according to an adapted version of a previously reported method.28 Briefly, a PCR was performed as described earlier, with the addition of 1 μCi of [α-32P]dCTP (NEN, Boston, MA; specific activity: 3,000 Ci/mmol). The reaction mixture (2 μL) was diluted 1:25 in 0.1% SDS plus 10 mmol/L EDTA, and further mixed 1:1 with a sequencing stop solution (95% formamide, 20 mmol/L EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). Samples were heated at 85°C for 5 minutes, chilled on ice, and immediately loaded onto a 5% acrylamide Tris-borate-EDTA (TBE) gel containing 10% glycerol. The gels were run at 4 to 8 W for 14 to 16 hours at room temperature. They were then fixed in 10% acetic acid and air-dried, and autoradiography was performed at −70°C with an intensifying screen for 2 to 12 hours.

**DNA sequencing of PCR products.** DNA sequencing of PCR products from the EBER region was performed on nine representative samples (cell lines B95-8, AG876, EB-3, and PT-LPD cases no. 1, 2, 3, 4A, 10, and 22). Briefly, a PCR was performed as described earlier. PCR products were purified on a 2% low-melting-point gel, and bands that contained the amplified DNA fragments were visualized under UV light and removed. Gel slices were treated with Gelase β-agarase and DNA fragments were recovered and purified by ethanol precipitation according to the manufacturer’s instructions (Epicentre Technologies, Madison, WI). These were then sequenced directly by the Sanger dye-termination method using an ABI 373A automated DNA sequencer (Applied Biosystems, Foster City, CA).

### RESULTS

**Analysis of EBNA-2 sequences.** Four EBV-infected cell lines, B95-8, Stork, AG876, and EB-3, were used as positive reference controls, and the HL60 promyelocytic leukemia cell line was used as a negative control in our analyses of the PT-LPD specimens. PCR amplification of sections of the EBNA-2 and EBNA-3c coding regions allowed us to distinguish between EBV types A and B on the basis of size differences in the products generated. EBNA-2 primers, which span a sequence that contains a 16-bp deletion in type
A EBV, generated the expected 168-bp fragment for B95-8, the prototype cell line infected by type A EBV, and for the Stork cell line. A 184-bp fragment consistent with infection by type B EBV was seen in the prototype cell line AG876 and in the EB-3 cell line (Fig 1A and Table 2). No cell line gave rise to both fragments, ruling out dual infection. To demonstrate the specificity of the PCR bands visualized by ethidium bromide fluorescence, the gels were transferred to nitrocellulose filters and the blots hybridized using an end-labeled internal oligonucleotide probe common to both EBV types (Fig 1B).

PCR analysis using the same primers was performed on the 27 PT-LPD specimens collected from 22 organ transplant recipients. The 168-bp DNA fragment found in EBV type A was generated in 24 cases (Fig 1A and Table 2). No 184-bp DNA fragments consistent with infection by type B EBV were seen in any cases. Three PT-LPD lesions (no. 11A, 15, and 17) were negative for EBNA-2. Successful amplification of portions of the p53 tumor-suppressor gene in each case confirmed that these samples contained DNA of suitable quality for PCR analysis. Southern blots of the PCR products hybridized to an internal probe common to both viral types confirmed these data (Fig 1B), which suggests that these three cases were free of EBV infection. These three cases previously had been found to be negative by Southern blot analysis using the EBV terminal repeat probe.22

Analysis of EBNA-3c sequences. EBNA-3c primers, which span a region that contains three deletions totaling 93 bp in EBV type A, generated the expected 153-bp fragment for B95-8 and Stork, consistent with infection by type A, and a 246-bp fragment, consistent with infection by type B, for AG876 and EB-3 (Fig 2A and Table 2). These primers generated the smaller A-type fragment in the same 24 PT-LPD samples that were shown to contain type A EBNA-2 (Fig 2A and Table 2). No 246-bp fragment consistent with infection by type B EBV was seen in any of the patient samples. The same three cases that were negative for EBNA-

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**Table 2. Results of EBNA-2, EBNA-3c, and EBER Analysis of EBV-Infected Cell Lines and PT-LPD Specimens**

<table>
<thead>
<tr>
<th>Sample</th>
<th>EBNA-2</th>
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<th>EBER Subtype</th>
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<td>A.i</td>
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<td>B</td>
<td>i</td>
<td>B.ii</td>
</tr>
<tr>
<td>EB-3†</td>
<td>B</td>
<td>B</td>
<td>i</td>
<td>B.iii</td>
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<td>A.iii</td>
</tr>
<tr>
<td>22</td>
<td>A</td>
<td>A</td>
<td>i</td>
<td>A.iii</td>
</tr>
</tbody>
</table>

*Type A EBV-infected cell line.
† Type B EBV-infected cell line.
‡ Negative.
2 (no. 11A, 15, and 17) were also negative for EBNA-3c. As for EBNA-2, no double bands suggesting dual infection were seen.

The specificity of the PCR bands was again confirmed by Southern blot analysis. Southern blots hybridized with a probe specific for type A EBV detected the two type A cell lines (B95-8 and Stork) and the 24 cases shown to contain type A EBNA-3c by ethidium bromide fluorescence. A probe specific for type B EBV was able to detect only the control type B cell lines AG876 and EB-3; the 24 PT-LPD samples appeared negative. Southern blots of the PCR products hybridized to both probes confirmed these data (Fig 2B).

**Analysis of EBER sequences.** The EBER region has been examined in previous studies to assess viral type.24,25,30 However, it has been noted that differences among the EBER genes of EBV isolates are less clearly type-specific than are differences among the EBNA genes.31 Therefore, we examined the EBER region to determine whether different EBER polymorphisms were present in our cases, which would confirm the presence of different EBV isolates, and to examine the relationship of such polymorphisms with EBV type. As the EBER primers span a 190-bp region that contains no deletions among various isolates, it is impossible to distinguish between EBER polymorphisms on the basis of electrophoretic fractionation of different-sized PCR products.

Therefore, SSCP analysis, which can reveal single nucleotide differences, was used to identify different EBER polymorphisms. Five different electrophoretic band patterns were identified by this technique. These five patterns and the EBER sequences they represented were designated i to v to avoid confusion with the existing EBNA type A and type B nomenclature. Three different electrophoretic band patterns (i to iii) were seen in our reference cell lines (Fig 3 and Table 2). Both Stork and B95-8 gave rise to the same pattern, which we designated pattern i: AG876 gave rise to a different electrophoretic band pattern, which we designated pattern ii; and EB-3 gave rise to a third band pattern, which we designated pattern iii.

SSCP analysis of the PT-LPD specimens with EBER primers gave rise to four different electrophoretic band patterns, most of which resembled one of the three patterns displayed by our reference cell lines (Fig 3 and Table 2). Interestingly, although 24 PT-LPD cases contained EBV type A EBNA-2 and EBNA-3c sequences, 19 of them displayed EBER band pattern iii seen in EB-3, one of the EBV type B control cell lines. Only three cases (no. 10, 12, and 16) exhibited EBER band pattern i seen in the two type A control cell lines. None of the cases displayed pattern ii seen in the EBV type B prototype cell line AG876. Cases no. 2 and 22 had band patterns iv and v, respectively, which did not match any of the control cell lines. The three cases that were negative for EBNA-2 and EBNA-3c sequences (no. 11A, 15, and 17) were also negative for EBER sequences.

The 190-bp EBER fragments generated by PCR in three of the cell lines and six representative PT-LPD cases were sequenced directly to determine the nature of the mutations causing the different SSCP electrophoretic band patterns. The samples sequenced included cell lines B95-8, AG876, and EB-3, and cases no. 1, 2, 3, 4A, 10, and 22 (Table 3). Cell lines B95-8 and AG876, which produced SSCP electrophoretic band patterns i and ii, respectively, matched their published EBER nucleotide sequences; these differ at
Fig 3. SSCP analysis of the EBER region of EBV-infected reference cell lines and 18 representative PT-LPD samples. Point mutations are visible as shifts in electrophoretic mobility, giving rise to three patterns among reference cell lines: i (Stork and B95-8), ii (AG876), and iii (EB-3). H2O and promyelocytic leukemia cell line HL60 serve as negative controls. Representative PT-LPD cases show two of these three patterns: i (cases no. 10, 12, and 16), and iii (cases no. 1, 3, 4A, 4B, 5, 6, 8A, 11B, 13, and 20). Two other patterns, iv (case no. 2) and v (case no. 22), are also seen. No pattern resembling that produced by control type B EBV cell line AG876 was seen among the 27 cases. Cases no. 11A, 15, and 17 are negative for EBER. ND, nondenatured DNA lane.

six nucleotide positions: 6808, 6884, 6886, 6911, 6927, and 6944. Cell line EB-3, which produced SSCP pattern iii, matched the nucleotide sequence for AG876 except for an A to G transition at position 6927. The same nucleotide sequence, containing this single mutation, was seen in those PT-LPD cases with SSCP band pattern iii (cases no. 1, 3, and 4A). Case no. 10, which produced SSCP pattern i, contained the expected nucleotide sequence for this fragment. Cases no. 2 and 22, which produced patterns iv and v, respectively, that did not match any of the controls, had unique nucleotide sequences. Case no. 2 (pattern iv) had nucleotide substitutions seen in pattern i at positions 6884, 6886, and 6927, and nucleotide substitutions seen in patterns ii and iii at positions 6808, 6911, and 6944. Case no. 22 (pattern v) had a pattern i sequence at all six nucleotide positions, but had a C to T transition at position 6836. Thus, the PT-LPD lesions showed marked heterogeneity with respect to the EBER region, in contrast to the consistency of type A EBV sequences in the EBNA genes studied.

DISCUSSION
The development of lymphoid neoplasms as a complication of organ transplantation has been well established since the original reports of McKhann and Penn et al. The association of EBV with their development was recognized by Hanto et al. and has been repeatedly confirmed. How-

Table 3. Sequence Analysis of EBER Region Amplified From Representative Cell Lines and Specimens

<table>
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<th>Sample</th>
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<th>Substitutions at Nucleotide Positions</th>
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<td></td>
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<td>T</td>
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<td>A</td>
</tr>
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<tr>
<td>22</td>
<td>v</td>
<td>T</td>
</tr>
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</table>

*Type A EBV-infected cell line.
†Type B EBV-infected cell line.
ever, until recently, little effort has been expended to examine
the EBV types involved in the pathogenesis of these lesions. We examined a panel of 27 PT-LPDs to determine
the frequency of type A and type B EBV in these lesions.
Since serologic and tissue-culture studies that have attempted
to determine the relative incidence of type A and type B EBV
infection in various EBV-associated diseases have generally
underestimated the degree of type B involvement, we relied on more sensitive DNA-based techniques, ie, PCR
amplification and SSCP analysis. Given the increased inci-
dence of type B EBV infection in immunocompromised
hosts and in neoplasms associated with immunocompromise,
and the brief report of type B EBV involvement in one PT-
LPD lesion, we expected type B EBV to be frequently
present in the PT-LPDs that we studied. We were therefore
surprised to find that all of the EBV-positive cases contained
only type A EBV, and that the PT-LPDs arising in transplant
recipients, despite their immunosuppressed state, do not fre-
quently harbor type B EBV.

Most previous studies of EBV type have relied principally
on analysis of the EBNA-2 locus, the site of greatest se-
quence divergence between EBV types A and B. However,
major differences between the two EBV families have been
shown in other genes expressed during latent cycle infection,
including EBNA-3a, 3b, and -3c. Moreover, deletions in
the EBNA-2 gene, which can render EBV typing at this
locus impossible, are not altogether uncommon. Therefore,
to confirm our findings by EBNA-2 analysis, we also exam-
ined the EBNA-3c gene. Twenty-four of 27 PT-LPD cases
(89%) contained EBV, consistent with the findings in other
studies. The EBNA-2 and -3c markers in these cases sege-
gated together in all EBNA-positive cases, consistently indi-
cating the presence of type A EBV. Interestingly, none of
the cases in our series contained type B EBNA-2 and -3c
sequences (Table 2). Dual infection, which has been sug-
gested to play a role in the pathogenesis of EBV-associated
diseases by some investigators, was not found in any of
the samples.

We also examined the EBER region, which codes for
products that are thought to control viral RNA processing
during latency, as this locus has been shown to vary among
different EBV isolates. However, the broad correlation ini-
tially described between the EBER region and type A or B
EBNA-2 sequences has not been consistently shown in stud-
ies of various cell lines and tumors. In almost one third of
published cases, EBNA data suggest the presence of one
EBV type and EBER data another EBV type. These dis-
crepancies are explained as mixed viruses arising by recom-
bination between type A and type B EBV in cases of dual
infection. Crossover is probably a fairly common event,
since the EBER and EBNA loci are separated by approxi-
ately 40 kb, about one quarter of the total length of the
EBV genome. However, such explanations ignore the
larger issue of whether the EBER locus is an appropriate
and accurate predictor of viral behavior. Since EBV type
was originally defined on the basis of EBNA gene products,
at least some of which have been clearly shown to play a
significant role in B-cell immortalization, and since the
EBER products have not been demonstrated to play a role
in this process, these hybrid cases suggested to us that the
EBER region would be unreliable as a marker for EBV type.

Nevertheless, polymorphisms at the EBER locus did allow
us to identify the presence of different EBV subtypes, inde-
pendent of the EBV family, A or B, to which they belong.
In all, five different EBER polymorphisms were identified
by SSCP analysis (Table 2). These were designated i to v
to avoid confusion with the existing EBNA nomenclature. One
EBER polymorphism (i) was identified in both type A con-
trol cell lines; two different patterns (ii and iii) were found
in the type B control cell lines. Interestingly, the third EBER
polymorphism (iii), found in one of the type B control cell
lines, was present in 19 of 24 EBV-positive cases (79%),
despite the fact that these were all type A by EBNA analysis.
Such frequent divergence between the EBNA and EBER
loci renders type analysis based on both markers difficult to
interpret and of questionable value. Therefore, while the
EBER locus can be useful to identify different EBV isolates,
these data confirm that it is unreliable as a marker for EBV
type, which we believe should be determined by sequence
deletions at the EBNA gene loci.

The majority of PT-LPDs contained type A EBV (Table 4) is consistent with what we know about the efficient
immortalization of B lymphocytes in vitro by type A EBV
and the importance of the EBNA-2 gene product in this
process. Whether the viral subtype (A.iii) present in most of
these cases is simply the most common one to which our
patient population is exposed, or whether it is particularly
well suited to the induction of these lesions remains to be
determined. If the A.iii subtype does in fact cause a sig-
ificantly higher rate of PT-LPD development than other forms
of EBV, it may be important to monitor patients who harbor
this subtype more closely for the future development of these
lesions.

Why no PT-LPD specimens in our series contained type
B EBV, despite the observation by others that transplant
recipients often harbor type B EBV in their peripheral
blood, is unclear. As pointed out earlier, the discrepancy
between these two findings may simply reflect a preponder-
ance of type A EBV in our area. However, this is unlikely
because approximately half of the AIDS-associated lympho-
mas studied in our laboratory contain type B EBV (unpub-
lished observation, August 1993), consistent with previous
reports. On the other hand, it may be that while type B
EBV can readily infect peripheral blood lymphocytes in
states of immunocompromise, they may be unable to effec-
tively induce the autonomous B-cell proliferation necessary

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of Cases</th>
<th>Type B</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.i</td>
<td>3</td>
<td>B.i</td>
<td>0</td>
</tr>
<tr>
<td>A.ii</td>
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<td>B.ii</td>
<td>0</td>
</tr>
<tr>
<td>A.iii</td>
<td>19</td>
<td>B.iii</td>
<td>0</td>
</tr>
<tr>
<td>A.iv</td>
<td>1</td>
<td>B.iv</td>
<td>0</td>
</tr>
<tr>
<td>A.v</td>
<td>1</td>
<td>B.v</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
for the development of PT-LPDs. Thus, peripheral blood infection by type A EBV (and perhaps by certain EBV A subtypes), which appear to have the capacity to induce these changes in B cells, may be much more relevant in the pathogenesis of the PT-LPDs. Retrospective peripheral blood analysis of the patients studied here, and prospective studies of our entire posttransplant patient population, are in progress, and should help to clarify these issues. However, regardles of the outcome of these studies, the presence of type A and not type B EBV in every EBV-positive PT-LPD studied here strongly suggests a pathogenic role for type A EBV in the development of these lymphoid proliferations.

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REFERENCES


Posttransplantation lymphoproliferative disorders frequently contain type A and not type B Epstein-Barr virus

D Frank, E Cesarman, YF Liu, RE Michler and DM Knowles