ASSOCIATION OF EPSTEIN-BARR VIRUS TYPES 1 AND 2 WITH ACQUIRED IMMUNODEFICIENCY SYNDROME-RELATED PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS

To the Editor:

It is with great interest that we read the recent article by Shibata et al reporting that 39 of 59 lymphomas (66%) present in lymph node biopsies obtained from individuals with the acquired immunodeficiency syndrome (AIDS) were associated with Epstein-Barr virus (EBV). Because EBV is capable of immortalizing B cells, there has been considerable speculation as to the role of EBV in a variety of lymphoproliferative malignancies, including AIDS-associated lymphomas.

There are two major subtypes of EBV, EBV type 1 and EBV type 2, distinguishable by the nuclear antigens expressed during latency (EBNA-2 and EBNA-3). EBV type 1 is more potent in inducing in vitro cell transformation; EBV type 2 is highly associated with endemic Burkitt's lymphoma.

Primary central nervous system (CNS) lymphomas are a distinct subset of AIDS-associated lymphomas, in that virtually all are (1) monoclonal, (2) infected with EBV, and (3) lack evidence of c-myc rearrangement. The high association with EBV prompted us to use the polymerase chain reaction (PCR) to determine if there was a preferential association with a specific EBV subtype. We analyzed 17 biopsies obtained from 14 AIDS patients, of which 7 (from 7 patients) were frozen tissue and 10 (from 7 patients) were buffered formalin-fixed paraffin-embedded specimens obtained at autopsy. Purified DNA was subjected to PCR using oligonucleotide primers specific for the EBNA 3C gene. The size of the amplified product permitted discrimination between EBV type 1 and EBV type 2 (153 bp v 246 bp, respectively); additional Southern analysis using an internal EBNA 3C probe was performed to verify the EBV subtype. Amplifications of HLA DQ was performed in parallel as an internal control for each reaction. All DNA was subjected to 30 cycles of PCR; DNA extracted from paraffin-embedded tissue underwent an additional 30 cycles of amplification after supplementation with additional Taq polymerase.

HLA DQ was amplified from all 17 specimens. EBV could be detected in 10 of the 17 specimens (59%) analyzed; 6 specimens contained EBV type 1 and 4 contained EBV type 2. One patient had 2 separate paraffin-embedded biopsies, of which EBV type 2 was amplified from only one of the biopsies. Retrospective microscopic evaluation of the biopsies showed that more tumor was present in the biopsy from which EBV DNA was amplified. The other 2 patients from whom 2 paraffin-embedded specimens were obtained had concordant PCR results on both specimens. Although variable success with amplification from DNA purified from paraffin-embedded tissue as compared with frozen tissue has been reported, such a biased effect was not apparent in this study (4 of 10 EBV negative from paraffin-embedded tissue v 3 of 7 EBV negative from frozen tissue).

Our results confirm the previously reported high association of EBV with primary CNS AIDS-associated lymphomas. Furthermore, we have shown that neither EBV subtype is preferentially associated with this subset of AIDS lymphomas. Our findings are consistent with the observations for non-CNS (ie, systemic) AIDS-associated lymphomas, in which 26% to 46% have been shown to be associated with EBV type 2. Lastly, the lack of preferential association of EBV subtype with primary CNS AIDS-associated...
lymphomas suggests that additional mechanisms (ie, lack of T-cell control, chronic antigenic stimulation, additional chromosomal events) play a significant role in the development of these malignancies.

Clifford J. Gunthel
Department of Medicine
Valerie Ng
Michael McGrath
Departments of Medicine and Laboratory Medicine
Brian Herndier
Departments of Laboratory Medicine and Pathology
Bruce Shiramizu
Department of Pediatrics
University of California, San Francisco
San Francisco, CA

REFERENCES
Association of Epstein-Barr virus types 1 and 2 with acquired immunodeficiency syndrome-related primary central nervous system lymphomas [letter; comment]

CJ Gunthel, V Ng, M McGrath, B Herndier and B Shiramizu