depleted in their 3 patients. T-cell chimerism before DLI, therefore, was mixed and not already predominantly of donor type, as in our cases.

Gardiner et al state that their technique using lineage-specific STR-PCR provides reliable results on the chimeric profile post-BMT and may be more useful than the FICTION assay in situations with low cell numbers. Although the important aspect of morphology and individual cell by cell analysis is lost with the PCR approach, we agree that STR-PCR is a most valuable tool in this context and therefore complementary to our FICTION analysis. However, we doubt that subtle changes in the degree of mixed chimerism can be seen with a semiquantitative technique according to which mixed chimerism is defined as a percentage of recipient cells between less than 90% and greater than 10%. Indeed, in the work of Gardiner et al, significant changes in every case were detected only concomitantly to the onset of clinical graft-versus-host disease (GVHD), a result similar to that obtained in our cases when using the AmpliType Polymarker PCR kit to document non–lineage-specific DNA chimerism. In contrast, when using our quantitative techniques, ie, FICTION and competitive differential bcr-abl RT-PCR as an additional disease-specific marker, the beginning of the critical switch period could be detected several weeks before the onset of clinical GVHD. Although we therefore very much support a larger scale study to better understand the kinetics of the GVL response after DLI in T-cell–depleted and non—T-cell–depleted BMT recipients, we strongly suggest the use of true quantitative measures of lineage-specific chimerism. The FICTION method is especially helpful in small centers, because it can be performed without larger equipment or in situations in which morphological control of the selected population is of additional value. Another attractive tool is the combination of FACS sorting and quantitative multiplex STR-PCR with fluorescent primers. This technique has been successfully applied by members of our group to study subtle changes of subset chimerism in patients undergoing nonmyeloablative stem cell transplantation and was shown to be predictive of response, GVHD, and disease recurrence.

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Detection of Human Herpesvirus-8 and Epstein-Barr Virus DNA in Primary Intraocular Lymphomas

To the Editor:

Two herpesviruses, human herpesvirus-8 (HHV-8) and Epstein-Barr virus (EBV), are able to contribute to lymphomagenesis in humans. HHV-8 is documented not only in a strong association with Kaposi’s sarcoma, but also in a high percentage of primary effusion lymphoma, Castleman’s disease, and multiple myeloma. Most recently, HHV-8 genome has been associated with primary central nervous system lymphoma and multiple myeloma. However, the role of HHV-8 in the pathogenesis of PCNSL remains controversial. In vitro, EBV efficiently transforms human B lymphocytes, causing them to proliferate continuously. EBV and the latent membrane protein can be detected in tumor cells of almost all AIDS-related PCNSL.

Primary intraocular lymphoma, a component of PCNSL, is a large B-cell, non-Hodgkin’s lymphoma. The disease is aggressive, with a 5-year survival rate of less than 33%. In the past 15 years, the incidence of the tumor has increased dramatically, coincident with the AIDS epidemic. We analyzed HHV-8 and EBV DNA sequences in 13 primary intraocular lymphoma specimens (3 of 5 eyes, 3 of 7 vitrectomies, and 0 of 1 vitreoretinal biopsy) showed typical large B-cell lymphoma (Fig 1). Of the 7 cases without obvious typical lymphoma cells, 5 (4 vitreous and 1 vitreoretinal biopsy) were also available for cytokine analysis and had high vitreous interleukin-10 (IL-10) levels with high ratios of IL-10 to IL-6, suggesting primary intraocular lymphoma.

On examination of the histology and cytology slides of the 13 cases, only 6 specimens (3 of 5 eyes, 3 of 7 vitrectomies, and 0 of 1 vitreoretinal biopsy) showed typical large B-cell lymphoma (Fig 1). Of the 7 cases without obvious typical lymphoma cells, 5 (4 vitreous and 1 vitreoretinal biopsy) were also available for cytokine analysis and had high vitreous interleukin-10 (IL-10) levels with high ratios of IL-10 to IL-6, suggesting primary intraocular lymphoma. Finally, 2 cases (1 eye with AIDS and 1 eye that had received radiation) showed only a few atypical lymphocytes in the subretinal space. The microdissected morphologically suspicious abnormal cells in all 13 cases had presented a rearrangement in FR3A of the IgH gene that confirmed the diagnosis of primary intraocular lymphoma (Fig 2).

Lymphoma cells of these 13 specimens, normal lymphocytes of 2 vitrectomy specimens, and the 2 uveitic cases were microdissected from either deparaffinized sections or cytological slides. DNA of these cells were extracted for polymerase chain reaction (PCR) amplification and Southern hybridization for HHV-8 or EBV genome. Multiple primers and positive and negative controls were also used to confirm the results. Four cases were positive for HHV-8 (Fig 2). Two were the AIDS-related lymphoma eyes and 2 were non-AIDS vitreous samples. The 2 non-AIDS patients were diagnosed with suspicious primary intraocular
lymphoma both clinically and cytopathologically. These 4 positive patients included 2 Americans and 2 French. Three samples (1 American AIDS eye and 2 vitreous specimens) were also microdissected to obtain nonmalignant cells (retinal and optic nerve cells from the eye and infiltrating lymphocytes from the other 2 vitreous specimens) for identification of the viral genome. HHV-8 DNA was undetectable in the inflammatory or ocular cells without tumor infiltration in the 3 early cases with primary intraocular lymphoma. EBV DNA was detected in only 1 of the 13 samples, ie, the French AIDS eye with a diffuse lymphoma. This investigation has shown HHV-8 DNA sequences in 2 of 2

Fig 1. Microphotograph showing typical intraocular lymphoma (arrow) in the subretinal space (A; case no. 8; insert, higher magnification of the lymphoma cells; R, retina; C, choroid) and a vitrectomy specimen (B; case no. 7; arrowheads, normal lymphocytes). (Original magnifications: A, hematoxylin & eosin, ×200, insert, ×400; B, Diff-Quick, ×400.)

IgH Rearrangement (FR3A)

HHV-8

EBV

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Fig 2. PCR amplification showing rearrangement of third framework region in the V\(\gamma\) region of heavy chain Ig gene in the lymphoma cells of all 13 cases, HHV-8 genome in 4 cases (lanes 3 and 4, AIDS patients; lanes 7 and 9, non-AIDS patients), and EBV genome in 1 case (lane 4, AIDS with lymphoma involving the orbit and eye). Lanes 1 through 13, cases no. 1 through 13, respectively; lane 14, negative control; lane 15, positive control.
AIDS-related intraocular lymphoma and in 2 of 11 non-AIDS intraocular lymphoma. The HHV-8 genome encodes homologs of cyclin D1, a cell-cycle control element; certain cytokines, regulators, and receptors; and Bcl-2, an antiapoptotic protein. The HHV-8 sequence also contains homologs of vial IRFs that may be involved in modulating both HHV-8 replication and HHV-8–associated tumorigenicity. The viral genome could therefore contribute to cellular growth and transformation through activation of the cell cycle. Although the virus could simply be an innocent passenger in this lymphoma, the so-far ubiquitous association of HHV-8 with AIDS-related PCNSL and relatively low incidence in non-AIDS patients, plus most other lymphoproliferative disorders in both AIDS and non-AIDS patients, strongly favor a causal role. In this series, HHV-8 was detected in 100% (2 cases: 1 American and 1 French) of AIDS-related intraocular lymphoma and in 1 of the 6 European and 1 of the 5 American, non-AIDS cases. Furthermore, HHV-8 DNA was found only in the neoplastic cells. We speculate that the nonneoplastic B lymphocytes in those AIDS patients who eventually develop primary intraocular lymphoma may be infected with HHV-8, HIV, or other viruses into the eye, in which ultimate transformation into malignant cells occurs. Another possibility may be the promotion of a second oncogenic virus.

Nine EBV genes are known to express as proteins in EBV-transformed B-lymphoblastoid cell lines. One of the latent membrane proteins of the EBV engages members of the tumor necrosis factor receptor-associated molecules and then activates NF-κB–driven expression of multiple viral and cellular genes. However, only certain subsets of AIDS-non Hodgkin’s lymphoma appear to be EBV-related, although a high frequency of EBV is reported in AIDS-PCNSL. Our data do not show a strong association between EBV and primary intraocular lymphoma, suggesting that EBV may not play a major role in the early development of primary intraocular lymphoma with or without AIDS.

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