Expression of Epstein-Barr Virus–Encoded Latent Membrane Protein 1 in Nonendemic Burkitt’s Lymphomas

To the Editor:

A restricted form of Epstein-Barr virus (EBV)-latent infection (latency I), has been consistently observed in Burkitt’s lymphoma (BL) cell lines and fresh BL biopsy specimens.1,2 This pattern of EBV latency is characterized by selective expression of EBV-encoded nuclear antigen (EBNA) 1 (together with two noncoding small RNAs—EBERs). The first demonstration of forms of EBV latency other than latency I in endemic BL has recently been provided by Niedobitek et al.3 who found unexpected heterogeneity in the latent gene phenotype of EBV+ BL biopsy specimens. Using sensitive immunohistologic techniques, expression of latent membrane protein (LMP) 1 was detected in a proportion of tumor cells in two cases and EBNA 2 was observed in some tumor cells in three other cases. LMP 1 expression was not detected in those cases displaying EBNA 2. In the remaining endemic BL cases EBV latent gene expression was restricted to the EBERs (latency I). These data conclusively show that EBV-infected tumor cells of endemic BL may sustain three distinct forms of viral latency. It is interesting to note in this context that the expression of LMP 1, in the absence of EBNA 2, has already been observed in a limited number of EBV+ acquired immunodeficiency syndrome (AIDS)-related BL cases.4,5
Table 1. EBV+ Nonendemic Burkitt’s Lymphomas Containing LMP 1-Expressing Tumor Cells

<table>
<thead>
<tr>
<th>No.</th>
<th>Age/Sex</th>
<th>HIV Infection</th>
<th>LMP 1</th>
<th>EBNA 2</th>
<th>CD30</th>
<th>BCL-2</th>
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<td>-</td>
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</tbody>
</table>

--, no expression; +, small numbers (<1%) of expressing tumor cells. Positive cells tended to display a focal distribution.

* Coexpression, as detected by double immunostaining.

In view of the heterogeneous phenotypic features observed in endemic BL cases, a further characterization of the pattern of EBV-latent gene phenotype in nonendemic BL cases would potentially be valuable. An additional phenotypic analysis of the BCL-2 oncoprotein and CD30 antigen expression in BL cells expressing LMP 1 or EBNA 2 may further clarify whether these events in vivo mirror those observed by some BL cell lines in vitro. To address this issue, we analyzed by EBER in situ hybridization and immunohistology a total of 66 biopsy specimens from 33 AIDS-related and 33 AIDS-unrelated BL, collected between 1984 and 1994. Out of this collection, we found 10 EBV+ AIDS-related and 6 EBV+ AIDS-unrelated BL cases. Human immunodeficiency virus (HIV)-test was performed in all patients. All the 16 cases were immunohistologically tested for LMP 1 (monoclonal antibody [MoAb] CS 1–4; Dako A/S, Glostrup, Denmark) and EBNA 2 (MoAb PE2; Dako) expression. The cases containing LMP 1 –expressing cells were also stained with the anti-CD30 MoAb BerH2 (Dako) and a BCL2-specific MoAb, 124 (Dako). Furthermore, a sensitive method for multiple immunohistochemical staining was performed to detect LMP 1 plus BCL2 or CD30.

In 3 of the 10 EBV+ AIDS-related BL and in 2 of the 6 EBV+ AIDS-unrelated BL cases, small numbers (<1%) of focally distributed tumor cells were found to express LMP 1 (Fig 1), but not EBNA 2 (Table 1), suggesting a latency II phenotype. LMP 1 expression was observed in both tumor cells with classic BL cell morphology or with immunoblastic-like morphology (enlarged nuclei with prominent nucleoli). The remaining EBV+ BL cases consisted exclusively of tumor cells showing an EBV phenotype corresponding to a latency I pattern. LMP 1+ and LMP 1– BL cases were histologically indistinguishable. Expression of BCL2 oncoprotein or CD30 in small numbers of tumor cells was observed in 4 of 5 and 1 of 5 LMP 1+ cases, respectively (Table 1). Double immunostaining showed BCL2 (but not CD30) coexpression in a fraction of LMP 1+ expressing cells (Fig 2). In all tested cases BCL2 expression was also observed in reactive small lymphoid cells.

Our study, which confirms LMP 1 expression in a fraction of EBV+ AIDS-related BL cases, also provides the first demonstration of a form of EBV latency other than latency I in a proportion of tumor cells of nonendemic AIDS-unrelated BL cases. These data, in conjunction with those of Niedobitek et al, document heterogeneous expression of EBV latent proteins throughout the entire spectrum of BL. Notably, in contrast with Niedobitek et al, who have failed to show an in vivo correlation between BCL2 and LMP 1 in endemic BL, we show here expression of both LMP 1 and BCL2 in tumor cells of nonendemic BL cases. A similar in vivo correlation has recently been described in EBV+ AIDS-related primary brain lymphomas. Such a phenotypic finding in vivo reflects phenotypic changes observed in vitro studies showing that BCL2 can be transactivated by LMP 1 in BL cell lines. Interestingly, the BCL2 expression induced by LMP 1 may protect tumor B cells from apoptosis and result in a higher proliferative rate.

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Fig 1. Expression of LMP 1 (red) in some tumor cells of a nonendemic AIDS-unrelated Burkitt’s lymphoma, using MoAb CS 1-4 and the APAAP technique. Original magnification × 400.

Fig 2. Same case as Fig 1. Coexpression of LMP 1 (red) and BCL2 (brown) in a large tumor cell. Sections were first immunostained with anti-LMP 1 MoAb and the APAAP method; then they were treated twice for 5 minutes in citrate buffer (pH 6) in a microwave oven to denature bound antibody molecules and to inactivate alkaline phosphatase present in the APAAP complex. Finally, sections were incubated overnight at 4°C with anti-BCL2 MoAb and immunostained by the ABC-peroxidase method. Hematoxylin counterstaining; original magnification × 630.
REFERENCES


Expression of Epstein-Barr virus-encoded latent membrane protein 1 in nonendemic Burkitt’s lymphomas [letter]

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