In situ demonstration of Epstein–Barr virus in intravenous drug abusers with generalized lymphadenopathy

F. Mampaso, C. Bellas, A. Molina, C. Quereda¹, T. Bricio and L. Buzon¹

Departments of Pathology and ¹Microbiology, Hospital Ramon y Cajal, Madrid, Spain

Summary: We have studied by the *in situ* hybridization method the presence of Epstein–Barr virus (EBV) DNA genome in lymph node tissues from 11 patients with persistent generalized lymphadenopathy. Using a biotinylated EBV DNA probe, we demonstrated EBV nucleic acid in scattered germinal centre cells in eight of the 11 cases. Our results suggest that EBV is not a determinant factor in the pathogenesis of this lymphadenopathy, but support its possible implication in B cell malignant transformation in cases of AIDS-associated lymphoma.

Introduction

Persistent generalized lymphadenopathy (PGL) has been increasingly observed among high risk groups for the acquired immune deficiency syndrome (AIDS).¹ Expression of RNA and antigens of human immunodeficiency virus (HIV) in lymph nodes from patients infected with HIV strongly support a role for HIV in the development and/or progression of this lymphadenopathy.² Several studies have pointed to a possible implication of other lymphotropic viruses, such as Epstein–Barr virus (EBV) in the pathological lesions found in patients with AIDS.³

Using the technique of *in situ* hybridization, we were able to demonstrate the presence of EBV genome in 8 of 11 lymph node tissues from intravenous drug abuser patients with PGL. The high incidence of EBV DNA hybridized positive signals encountered in our group of patients could be of interest in obtaining information regarding the possible implication of EBV in the pathogenesis of AIDS-associated lymphoma.

Patients and methods

All 11 patients were intravenous drug abuser–HIV-infected young men referred to our hospital for evaluation of PGL. For this study, PGL was defined as lymphadenopathy involving more than two extra-inguinal sites of at least 3 months

duration with fever, diarrhoea, sweating and weight loss. The EBV serology was assessed at the time of lymph node biopsies by determining viral capsid antigens, early antigen complex and EBV nuclear antigen titres. In addition, several normal lymph nodes derived from different sites (autopsy material) were obtained from seven patients without clinical, serological and histological evidence suggestive of EBV infection.

Lymph node biopsy specimens were fixed in 10% buffered formaldehyde. Routine histopathology (H & E, PAS, Giemsa and reticulin) was performed on paraffin-embedded tissues. All the material was serially cut and 4 µm thick sections were mounted on to glass slides coated with 50 µg/ml of Poly-L-Lysine (Sigma Chemical Co., St Louis, MO, USA). The sections were treated with Proteinase K (Sigma) solution, 0.01 mg/ml in phosphate-buffered saline, pH 7.4, at 37°C for 5–10 minutes. Hybridization was done as described elsewhere.⁴ Briefly, sections were covered with 20 µl of the hybridization cocktail containing biotinylated EBV DNA probe (Bioprobe; Enzo Diagnostics Inc., New York, NY, USA). The probe used was the BamHI 'V' (internal repeat 1) region of the EBV genome cloned into pBR322 containing a 3.1 kb sequence which is reiterated 11–14 times in the EBV genome. The sections were then covered with a cover slip and after denaturation by heating to 92°C on a hot plate for 8 minutes, hybridization was allowed to take place at 37°C for 3 hours. The reaction was visualized after incubation with avidin–biotin–peroxidase complex (Enzo) with the use of hydrogen peroxide and aminoethyl carbazole (Sigma). Controls included the omission of the specific viral probe (EBV) and the replacement

Correspondence: Francisco Mampaso, M.D., Department of Pathology, Hospital Ramon y Cajal, Carretera de Colmenar km 9.100, 28034-Madrid, Spain.

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of the EBV probe by biotinylated cytomegalovirus (Enzo). An oral hairy leukoplakia lesion from an HIV patient as well as the EBV-infected cell line B95-8 were used as positive controls.

Results

All lymph nodes from intravenous drug abuser –HIV-infected patients were found to be enlarged and showed histological alterations compatible with the definition of PGL. The lymph node lesions were classified as follicular hyperplasia with or without severe follicular fragmentation and follicular involution. The histological alterations described as well as the serological and *in situ* hybridization studies are shown in Table I.

EBV DNA-positive reaction, detected in eight of 11 cases, exhibited a round and homogeneous nuclear pattern, frequently surrounded by a clear halo. These positive signals showed differences in size, with the use of a high-power objective being required to identify some extremely small nuclear hybridized reactions. The majority of the EBV genome was detected in the germinal centre cells, but also observed in scattered lymphoid cells of the mantle zone and occasionally, in the small lymphocytes of the interfollicular area (Figure 1). None of the lymph node specimens without clinical, serological or histological evidence of EBV infection showed EBV DNA-positive signals.

Discussion

PGL is considered an early HIV infection stage, in most cases prodromic to AIDS. It is characterized by the existence of activated and proliferative germinal centres, which are the place for B cell maturation and proliferation. In addition, these will be the possible site of malignant transformation to a B cell lymphoma (the most frequent lymphoid neoplasia affecting AIDS patients).⁵ The data available suggest that HIV-infected patients

Figure 1 Positive hybridization in the nuclei of follicular centre cells and mantle cells (insert), using a biotinylated EBV DNA probe ($\times 1,000$).

are easy targets for EBV reactivation.⁶ Our serological findings indicate a previous EBV infection in all patients. None of the early antigen complex positive cases presented the Burkitt's lymphoma or nasopharyngeal carcinoma serological patterns.

In this study we have demonstrated the presence of EBV DNA genome in the lymphoid cells of lymph nodes from 8 out of 11 patients with PGL. These findings are in contrast to those of Uccini *et al.*,⁷ who have recently reported the absence of EBV DNA sequences in patients with PGL. It is possible that some differences might exist in the hybridization method procedure. Another and perhaps more likely explanation for our findings, might be the extremely symptomatic and more advanced HIV disease of our patients.

We think that, in spite of the presence of EBV DNA in the majority of the lymph nodes involved by PGL, as demonstrated in this study, the fact that it was not found in all our cases, suggests that this viral genome is not a determinant factor in the pathogenesis of PGL. Nevertheless, our findings

Table I Histological, serological and genomic findings in 11 patients infected with HIV with persistent generalized lymphadenopathy

	Number of cases	EA	EBV serology		EBV DNA in situ
			VCA	EBV-na	
Follicular hyperplasia	1/11	0/1	1/1	0/1	1/1
Follicular fragmentation	6/11	3/6	6/6	0/6	6/6
Follicular involution	4/11	0/4	4/4	0/4	1/4
Total	11/11	3/11	11/11	0/11	8/11

EBV = Epstein–Barr virus; EA = early antigen complex; VCA = viral capsid antigen.

could also support the hypothesis that EBV may be able to induce oligoclonal proliferations of B cells, thus favouring c-myc translocation and neoplastic transformation.⁸

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