Lack of correlation between expression of Epstein-Barr virus (EBV) latent membrane protein and bcl-2 oncoprotein in vivo

Q Tao, G Srivastava, S L Loke, F C S Ho

Abstract

Aims—To evaluate whether there is any correlation between the expression of Epstein-Barr virus (EBV) latent membrane protein (LMP) and oncoprotein bcl-2 in the lymph node biopsy specimens of a Chinese patient with EBV-related reactive lymphoproliferation who later developed T cell lymphoma after a short period of time.

Methods—Immunohistochemistry, with a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) method and New Fuchsin as a chromogen, was used for single staining of bcl-2 or LMP. Double immunostaining combining APAAP and indirect immunofluorescence was performed for dual labelling of LMP and bcl-2.

Results—bcl-2 was expressed in 10–30% of cells in the first lymph node biopsy specimen (EBV-associated lymphoproliferative disorder) and 30–50% of cells in the second lymph node biopsy specimen (T cell lymphoma). LMP was expressed in the first biopsy specimen but not in the second. Double immunostaining results showed that around 78% of LMP positive cells were bcl-2 negative and 94% bcl-2 positive cells were LMP negative. Among the very small fraction of LMP and bcl-2 double positive cells, the intensity of bcl-2 staining was heterogeneous and was not always stronger than that observed in LMP negative bcl-2 positive cells.

Conclusions—The expression of bcl-2 protein is independent of LMP protein status in vivo. Several mechanisms may be involved in EBV associated lymphomagenesis, and bcl-2 induction may occur independently of LMP expression.

Case report

A 58 year old Chinese man presented initially with generalised lymphadenopathy. A lymph node biopsy specimen showed disturbed architecture with a preponderance of large B blasts mixed with numerous CD8+ T lymphocytes, consistent with an acute EBV infection. Immunohistochemical staining for κ and λ light chains and gene rearrangement studies confirmed the absence of clonal T or B cells. Polyclonal episomal and linear EBV genome,
consistent with lytic infection, was detected by Southern blot analysis. The histological features of the lymph node biopsy specimen resembled those of a reactive lymphoproliferative lesion. After biopsy lymph node enlargement spontaneously regressed, but lymphadenopathy recurred eight months later, and the second lymph node biopsy specimen showed T cell lymphoma, confirmed by detection of clonally rearranged T cell receptor β chain gene on Southern blot analysis. However, EBV genome could not be detected in the second biopsy specimen by Southern blot hybridisation and also by in situ hybridisation using fluorescein isothiocyanate (FITC) conjugated oligomer probe for EBV encoded EBER RNA (Dako Japan Co. Ltd). The patient died of hepatic encephalopathy five months after chemotherapy. A necropsy was not performed.

Methods

For immunohistochemistry, a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) method was used on cryostat sections. New Fuchsin was used as substrate. Normal rabbit serum or anti-chromogranin were used as negative controls. Cytospin slides of EBV infected Raji cell line (latent), p3HR1, and B95–8 (lytic) were used as positive controls for bcl-2 or LMP. Double immunostaining was performed essentially as described by Ramshaw and Parums10 by combining APAAP and indirect immunofluorescence, but using New Fuchsin as substrate instead of Fast Red for APAAP.

Results

Strong expression of LMP (using CS. 1–4 monoclonal antibody, courtesy of Dr M Rowe) was detected in about 15% of cells in the first lymph node biopsy specimen (EBV related reactive lymphoproliferation) by immunostaining. bcl-2 protein was also detectable in 10–30% of total cells (using antigen to bcl-2 antibody, courtesy of Dr D Mason) (fig 1A). Using a double immunostaining method, we were able to study the association between the expression of these two proteins. Results showed that there was no correlation between the expression of LMP and bcl-2. Around 78% of LMP positive cells were bcl-2 negative and 94% bcl-2 positive cells were LMP negative (fig 2). Even among the very low percentage of both LMP and bcl-2 positive cells, the staining intensity of bcl-2 also varied from weak to strong and these LMP and bcl-2 positive cells did not always have stronger staining than that of LMP negative bcl-2 positive cells. This pattern is similar to the staining of bcl-2 and LMP in a Raji cell line. bcl-2 protein was still detectable in 30–50% of cells in the second tumour biopsy specimen in which EBV could not be detected, and there were no differences in the staining intensities of bcl-2 between the first biopsy specimen and the second lymphoma biopsy specimen (fig 1 B).

Discussion

bcl-2 is expressed in a series of normal and neoplastic lymphoid tissues. High non-physiological concentrations of LMP may induce the expression of bcl-2 in vitro. However, the expression of LMP was rarely followed by bcl-2 expression in vivo in our case. Moreover, lack of correlation between the expression of LMP and bcl-2 in Hodgkin’s disease14 and nasopharyngeal carcinomas15 has been described recently. Martin et al have also recently reported the lack of correlation between LMP and bcl-2 in human B cells which were transfected in vitro with LMP expression vectors.16 Furthermore, no significant correlation between the expression of LMP and the activation markers CD23 or CD25 was observed in the first biopsy specimen of the patient described in this report (Q Tao, unpublished results), which agrees with

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**Figure 1** Immunostaining for bcl-2 protein using alkaline phosphatase anti-alkaline phosphatase (APAAP) on cryostat sections. (A) first biopsy specimen (EBV related reactive lymphoproliferation); (B) second biopsy specimen (EBV negative T cell lymphoma).
the recent reports by other groups. The life-long persistence of EBV in seropositive subjects is unlikely to depend on the long term survival of EBV positive cells with high expression of bcl-2 induced by LMP. Conversely, cells which express LMP at high concentrations will provide antigenic sites recognisable by cytotoxic T cells and would be removed rapidly. Other mechanisms for life-long persistence may be more important, including latent infection by EBV in epithelium and lymphocytes with periodic activation of a lytic infection cycle, leading to a reservoir of latently infected cells.

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