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

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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.**

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The *bcl*-2 proto-oncogene is located on the long arm of chromosome 18 (18q21).[1] In follicular lymphomas the expression of *bcl*-2 mRNA is deregulated through the 14;18 translocation with juxtaposition of *bcl*-2 gene with the immunoglobulin heavy chain locus on chromosome 14.[1] However, increased expression of *bcl*-2 oncoprotein is not dependent on the presence of a 14;18 chromosomal translocation.[2] The *bcl*-2 protein is also detectable in normal lymphoid tissues, and expressed in a wide range of neoplastic and lymphoproliferative diseases.[3] Abnormally high expression of *bcl*-2 has been associated with resistance to apoptosis.[4]

Epstein-Barr virus (EBV) is associated with several malignancies, including nasopharyngeal carcinomas,[5] Burkitt's lymphomas,[6] Hodgkin's disease,[7] and lymphomas occurring in immunocompromised[8] or organ transplant recipients.[9] Gregory *et al* have shown that the activation of EBV latent genes protects human B cells from death by apoptosis.[10] Recently, Finke *et al* reported that the expression of *bcl*-2 protein can be transactivated by transfection of EBV negative Burkitt's or non-Hodgkin's lymphoma cell lines with EBV latent membrane protein (LMP) gene carrying expression vectors.[11] Moreover, Henderson *et al* have also reported that the induction of *bcl*-2 expression by LMP-1 protects B cells from apoptosis.[12] Based on these results, Finke *et al* have postulated that high expression of *bcl*-2 induced by EBV is important for the long term survival of EBV positive cells in seropositive subjects, and proposed that rescue from apoptosis of lymphocytes by EBV through induction of *bcl*-2 might be a first important step in the tumorigenesis of certain lymphomas in vitro. However, it should be pointed out that all these studies have been carried out using in vitro systems in which LMP gene carrying retroviral vectors were transfected to produce supraphysiological concentrations of LMP proteins in EBV-negative Burkitt's or non-Hodgkin's lymphoma cell lines. We therefore ask whether a similar correlation also exists between the expression of LMP and *bcl*-2 in vivo. Here we report our observation about the expression of LMP and *bcl*-2 in a Chinese patient with EBV related reactive lymphoproliferation who later developed EBV negative T cell lymphoma after a short period of time.

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.

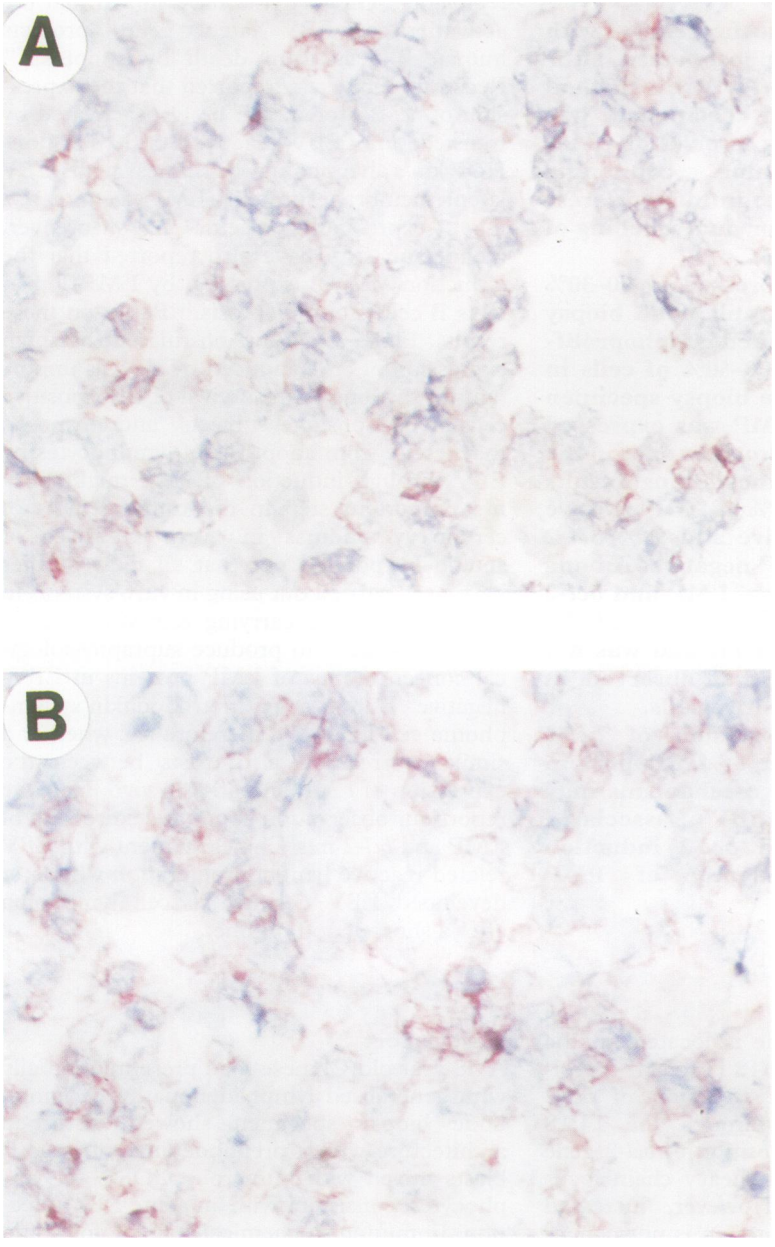

Figure 1 Immunostaining for bcl-2 protein using alkaline phosphatase anti-alkaline phosphatase (APAAP) on cryostat sections. (A) first biopsy specimen (EBV related reactive lymphoproliferation); (B) second biopsy specimen (EBV negative T cell lymphoma).

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 Parums[13] 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 disease[14,15] and nasopharyngeal carcinomas[16] 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.[17] 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

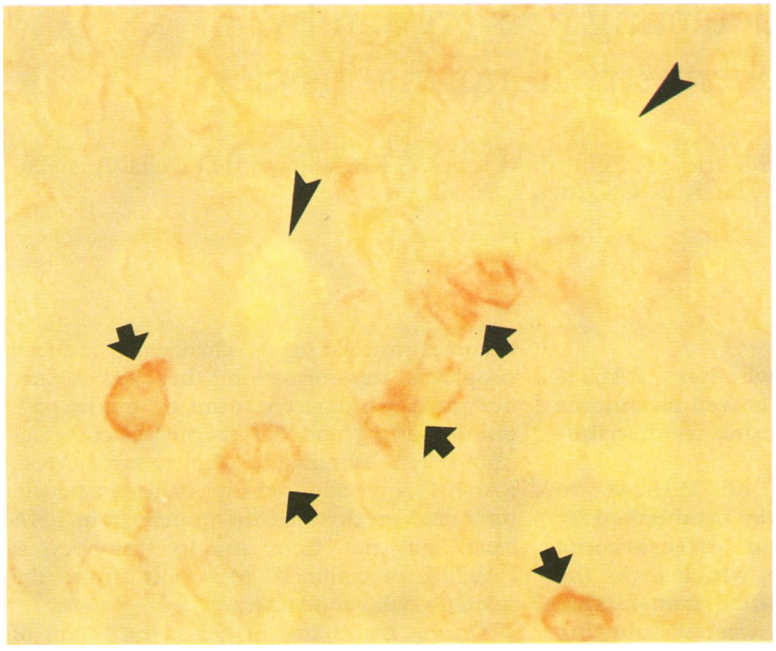

Figure 2 Double immunostaining combining APAAP for bcl-2 protein and indirect immunofluorescence (FITC) for LMP on the cryostat section of the first biopsy specimen (EBV-related reactive lymphoproliferation). Double exposure of the transmitted bright field illumination and FITC immunofluorescence is shown. Arrow: LMP negative and bcl-2 positive cells; arrowhead: LMP positive and bcl-2 negative cells.

the recent reports by other groups.[14 18] 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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