RAPID COMMUNICATION

High Expression of the bcl-x Gene in Reed-Sternberg Cells of Hodgkin’s Disease

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The expression of bcl-x protein, a bcl-2–related protein present in cortical thymocytes, activated lymphocytes, and plasma cells of reactive lymph nodes, was investigated in 44 cases of Hodgkin’s disease (HD) in parallel with bcl-2 and Epstein-Barr virus (EBV) status. Eighty-six percent of the cases were positive for bcl-x, among them 27% with a strong signal in more than 75% of the Reed-Sternberg cells. Positivity for bcl-x was found in, respectively, 100% and 92% of the nodular sclerosis and mixed cellularity subtypes, although 4 cases of lymphocyte predominance subtype were negative. This finding was in contrast with the weaker positivity for bcl-2 staining in 44% of the cases. EBV small RNAs were detected in 43% of the cases by using in situ hybridization. Of interest, 100% of the EBV-positive samples were positive for bcl-x, whereas only 38% of these cases were bcl-2 positive. Our findings show that the bcl-x gene expression is high in HD, suggesting that bcl-x may have a role in the pathogenesis of at least some cases of HD via apoptosis regulation.

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MATERIALS AND METHODS

Study design and patients’ profile. Forty-four cases of HD were retrospectively analyzed for immunohistochemistry. The tissues were fixed in 10% formalin, embedded in paraffin, and sectioned at 3-μm thickness. The distribution of cases according to subtype was as follows: 4 lymphocyte predominance (LP), 14 nodular sclerosis (NS), and 26 mixed cellularity (MC) (Table 1).

Immunohistochemistry. bcl-x polyclonal antisera (no. 1695) were raised against peptides corresponding to amino acids 46 to 50 of the human bcl-x protein and have been described in detail elsewhere. bcl-2 monoclonal antibody was a gift from Dr. D.Y. Mason (Oxford, UK). The standard immunophenotype of RS cells was performed on paraffin sections according to Chittal et al. Antibodies were shown using the streptavidin-biotin complex StreptABCComplex/HRP Duet (DAKO, Glostrup, Denmark; K692) on routinely processed paraffin sections with prior antigen retrieval using microwave heating. Assessment for positivity was described as follows: negative if less than 1% of tumor cells were positive; +, if 1% to 50% tumor cells were positive; ++, if 50% to 75% tumor cells were positive; and ++++, if greater than 75% tumor cells were positive.

In situ hybridization. In situ hybridization for EBV detection has been described elsewhere. Briefly, the DAKO hybridization

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RESULTS

bcl-x reactivity was first tested on two reactive lymph node specimens as a control. Plasma cells, large cells within the interfollicular area (activate lymphocytes presumably), and a few scattered lymphocytes within germinal centers were stained as previously reported (data not shown).

Among the 44 cases with HD, 38 (86%) were found to be positive for bcl-x staining (Table 1 and Figs 1 and 2). A positive internal control for each case was the immunostaining of plasma cells. Of the 43 cases tested for bcl-2 immunostaining, 19 (44%) were positive (Table 1 and Fig 3). Small reactive lymphocytes were positively stained and served as a positive internal control for bcl-2. In general, the intensity of bcl-x staining of RS cells was stronger than bcl-2. In addition, the percentage of RS cells that stained positive for bcl-x was also generally greater than for bcl-2. For example, 12 cases (27%) had more than 75% of RS cells stained for bcl-x, whereas in no case was bcl-2 staining of RS cells found as frequently (Table 1). EBV small RNAs were detected in 19 of 44 (43%) of the cases by in situ hybridization with EBER oligonucleotides. The signal was clearly nuclear in each case and abolished by RNaseA but not by DNase I pretreatment, as previously reported (data not shown).

None of the four LP-HD samples were stained by either bcl-x nor bcl-2 antibodies (Table 1). In contrast, 14 of 14 (100%) of NS-HD and 24 of 26 (92%) of the MC-HD stained for bcl-x antibodies (Table 1). For bcl-2, 11 of 14 (78%) of the NS subtype cases and 9 of 25 (36%) of the MC subtype cases were bcl-2 positive (Table 1). When bcl-x and bcl-2 immunostaining were related to EBV status, 19 of 19 of the EBER-positive samples were positive for bcl-x, whereas only 7 of 18 (38%) of the cases were bcl-2 positive.

DISCUSSION

Although HD is a well-defined clinical entity, its pathogenesis and cellular origin remain an enigma. The RS cells constitute only 1% or less of the total cell population in the tumor, with the remainder consisting primarily of reactive benign cells. The findings that the t(14;18) translocation, a genetic event described in follicular B-cell lymphoma, was present in HD tissues supported a B-cell origin for HD.

Nevertheless, this result raised a controversy because other groups failed to detect the t(14;18) translocation in HD by using the same method. bcl-x, a bcl-2-related protein, has recently been shown to be expressed in human lymph nodes, especially in plasma cells and in some activated lymphocytes. This distribution differs strikingly from bcl-2, which is usually found in the majority of resting long-lived memory lymphocytes located in the mantle zone and in many interfollicular lymphocytes. We have shown in this study that bcl-x is strongly associated with the RS cells of HD in most cases (86%), allowing for the possible use of bcl-x immunostaining as a new tool for RS cell immunohistochemistry.

Immunoblot analysis of bcl-x has been previously performed in a wide variety of normal tissues, demonstrating that in all tissues examined, including in lymph nodes, the bcl-x-L protein was predominantly detected, whereas the bcl-x-S protein was far less frequent. In HD samples, in which the tumor cells are rare, it may be difficult to address this question by either immunoblot or reverse transcriptase-polymerase chain reaction. Furthermore, monoclonal antibodies specific for the two forms of the bcl-x protein are not yet available and may be difficult to obtain, because the bcl-x-S protein differs from bcl-2 and bcl-x-L in that an internal region of 63 amino acids, which is highly homologous among members of the bcl-2 protein family, has been deleted.
Fig 1. Case of MC-HD positive with anti-bcl-x antibody. Note that the germinal center of the lymphoid follicle is not labelled (arrows) and that, as expected, the plasma cells are positive (arrowheads). (Hematoxylin counterstaining, peroxidase-diaminobenzidin; original magnification ×300.)

Fig 2. Case of MC-HD in which virtually all tumor cells (RS cells and mononuclear variants) are labeled. Note that the labeling is heterogeneous and varies greatly from cell to cell. (Hematoxylin counterstaining, peroxidase-diaminobenzidin; original magnification ×300.)

Fig 3. Strong immunostaining observed in one case of HD with anti-bcl-2 antibody. Note that, similar to bcl-x, the pattern of the staining is heterogeneous. Small lymphocytes served as an internal positive control. (Hematoxylin counterstaining, peroxidase-diaminobenzidin; original magnification ×400.)
by alternative splicing of the primary bcl-x mRNA transcript. Whatever the type of the bcl-x protein present in the RS cells, the finding in the same HD samples of bcl-x and bcl-2 (19 of 44 or 43%) raises the possibility of interaction between these two proteins within RS cells, because Sato et al recently showed interactions between bcl-2 and the bcl-x proteins by using a yeast two-hybrid system. Furthermore, another bcl-2 homology, mcl-1, has also been detected in RS cells and has been shown to be capable of binding to bcl-x-L. Although not examined here, other proteins such as the recently described bad (bcl-2/bcl-x-L associated death promoter) might be involved in the regulation of cell death in RS cells, because it has been shown to bind to bcl-x.

bcl-x immunodetection was found to be more frequent in EBV-positive cases, with 100% of the tumors being positive. Because EBV LMP1 has been reported to upregulate bcl-2 expression in vitro and to protect EBV-infected B cells from programmed cell death, it is tempting to speculate that the bcl-x gene may also be upregulated by the same mechanism. Moreover, open reading frames within the EBV gene BHRF1 possess homology to bcl-2 and BHRF1 overexpression has been reported to block apoptosis. This finding raises the possibility that EBV BHRF1 might recognize other members of the bcl-2 family including bcl-x.

In conclusion, we have found that bcl-x gene expression is high in RS cells of HD, especially the NS and MC subtypes and in EBV-positive tumors, suggesting that apoptosis-regulatory proteins may have a role in the pathogenesis of HD.

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