ASSOCIATION BETWEEN B-TYPE EPSTEIN-BARR VIRUS AND HODGKIN’S DISEASE IN IMMUNOCOMPROMISED PATIENTS

To the Editor:

A high frequency of Epstein-Barr virus (EBV)-positive Hodgkin’s disease (HD) is observed in patients with human immunodeficiency virus (HIV) infection.1 Boyle et al,2 in this journal, reported the presence of B-type EBV in 1 of 2 HIV-positive HD cases, as well as in an additional immunocompromised HIV-negative HD case. These data suggest that B-type EBV, which is considered to be a much less potent transformer of lymphocytes than A-type virus strain, may be pathogenetically involved in HIV-associated HD, as well as in HD arising in other conditions of immunodeficiency. Similar considerations have been previously drawn from studies on EBV subtypes in EBV-positive non-Hodgkin’s lymphomas.3,4

We recently used polymerase chain reaction (PCR) to characterize EBV subtypes in 30 EBV-positive HD samples from 10 HIV-positive as well as from 20 HIV-negative Italian patients, all tumor samples harboring the EBV genome in Hodgkin’s and Reed-Sternberg (HR-S) cells.5 B-type EBV was detected in 5 of 10 HD samples from the HIV-positive group, but only in 1 of 20 HD samples from the HIV-negative group (a 61-year-old woman who had developed an acute thrombocytopenia 6 months before HD was diagnosed). In 1 of the 5 B-type EBV positive HD samples from HIV-infected patients, a concomitant A-type EBV positivity was demonstrated by PCR and confirmed by DNA sequence analysis. However, in such a case we cannot ascribe a possible pathogenetic role in the disease to A-type or B-type, or to both EBV strains, due to the impossibility to type the virus strain in the HR-S cells.

These results from a larger series of Italian patients with HD are fully concordant with those reported by Boyle et al; in addition, they exclude that geographic factors might have favored B-type EBV-induced lymphoproliferation.

On the other hand, Boyle et al observed weak signals of both EBV types by PCR in some HIV-unrelated EBV-negative HD samples, suggesting that the immunocompromise related to HD or induced by the treatment might increase the number of EBV-infected peripheral blood lymphocytes (PBLs).

We did not observe such a pattern in an additional series of 30 HIV-unrelated EBV-negative HD samples. Seven of the samples were EBV-positive by PCR (using primers specific for the IR3 sequence and common to both EBV types), but EBV-negative in HR-S cells by sensitive in situ hybridization (ISH) assays (EBERs) as well as by LMP-1 immunohistochemistry. B-type EBV positivity
was never detected in these 7 samples (5 cases carried A-type EBV, while the other 2 cases were repeatedly PCR-negative for both EBV types using EBNA 2 specific primers). Positivity restricted to putative bystander lymphocytes in HD lesions was ascertained by ISH assay (EBERs) in three samples.

Differences in PCR sensitivity might account for our inability to detect B-type EBV-infected lymphocytes, if any, in our samples. In any case, further studies should specifically investigate PBLs from both healthy and immunocompromised individuals to determine whether different conditions of immunodeficiency may favor B-type EBV infection of such lymphocytes and the development of B-type EBV-related lymphoproliferations.

REFERENCES


RESPONSE

We would like to clarify some of the issues contained in a letter that appeared in the June 15, 1993 issue of BLOOD (81:3480, 1993). This letter was a response to our recent report8 and to a related publication by Drach et al13 in the same issue of BLOOD.

The aim of our study was to document the existence of functional P-glycoprotein in normal human lymphocytes and to characterize the distribution of P-glycoprotein in different lymphocyte subsets. We did not use the term “overexpression” in any of our conclusions, as this term would only be applicable to genetic or epigenetic variants derived from a specific cell line. It also was not a goal of our report to discuss the degree of drug resistance that would be conferred by P-glycoprotein in normal lymphocytes or to compare the level of P-glycoprotein between these cells and any established multidrug-resistant cell lines. The information relevant to the latter issue, however, has been presented elsewhere.

We have previously reported14 that the relative amount of MDR1 mRNA (normalized by the level of β2-microglobulin mRNA) in the lymphoid population of normal bone marrow cells, consisting mostly of mature lymphocytes, is similar or slightly lower than that of a multidrug-resistant carcinoma cell line, KB-8-5.4 Essentially the same result was obtained with peripheral blood lymphocytes (our unpublished data). We do not know how the level of MDR1 mRNA in the DOX 6 cell line, used as a positive control by Drach et al,5 compares with the KB series of multidrug-resistant cell lines that we usually use as standards for MDR1 mRNA expression. Our RNA measurements are in general agreement with the report of Coon et al,5 who showed that the rate of rhodamine 123 efflux by CD8+ lymphocytes was intermediate between that of KB-8-5 and its immediate predecessor, KB-8 cell line. KB-8-5 cells show a 3.2- to 6.3-fold increase in drug resistance over their original unselected precursor, KB-8-3-1 cell line.4 However, these cells contain only 3 to 5 molecules of MDR1 mRNA per cell, and the less resistant KB-8 cell line has approximately one MDR1 mRNA molecule per cell.4 It is not particularly surprising that similar low levels of MDR1 mRNA in peripheral blood cells were below the limit for reliable detection in your filter hybridization assays.7

We would also like to point out that P-glycoprotein-mediated multidrug resistance shows the best correlation with the density of P-glycoprotein in the cell membrane, and not with the absolute amount of P-glycoprotein per cell.8 Thus, one can expect that the amount of P-glycoprotein, as measured by immunofluorescence in a lymphocyte, may correspond to a much higher P-glycoprotein level in the larger epithelial or blastoid cells with a similar degree of drug resistance. In practical terms, it is difficult to normalize the cell surface area when comparing cell populations of very different size and tissue origin. Therefore, one would need to use a set of lymphocytic cell lines with known levels of multidrug resistance as standards to correlate the absolute levels of P-glycoprotein, measured by immunofluorescence or immunochemistry in lymphocytes, with the anticipated levels of resistance to P-glycoprotein-transports drugs. Because no such lymphocytic standards are available to us, we have refrained from inferring any specific level of drug resistance from the immunofluorescence intensity of normal peripheral blood lymphocytes.

Finally, we would like to comment on the suggestion to confirm the findings reported in our1 and Drach et al's8 papers by in vitro progenitor assays. We have already reported the results of both clonogenic and long-term culture initiating progenitor assays for human bone marrow lymphoid cells, separated on the basis of rhodamine 123 efflux or P-glycoprotein immunofluorescence. Both types of progenitors were predominantly associated with the P-glycoprotein–positive cell population.9

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REFERENCES

Association between B-type Epstein-Barr virus and Hodgkin's disease in immunocompromised patients [letter; comment]

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