EDITORIAL

Epstein-Barr Virus-Carrying Cells in Hodgkin's Disease

By George Klein

EPSTEIN-BARR VIRUS (EBV) plays a causative role in infectious mononucleosis and in the immunoblastic lymphoproliferative diseases that arise in certain congenital and acquired immunodeficiencies. High endemic Burkitt lymphoma (BL) is EBV-DNA positive in 97% of the cases. EBV genomes were recently also found in a number of T-cell-derived leukemias and in the T-cell-derived lethal midline granuloma. The virus is most consistently associated with nasopharyngeal carcinoma (NPC). Low differentiated and anaplastic NPC carry EBV genomes in virtually 100% of the cases.

The idea that the virus plays an important role in the genesis of BL and NPC has originally come from serologic studies. African BL and NPC of any geographic origin was always found in EBV-seropositives. The small EBV-seronegative subpopulation was uniquely absent in these diseases and most patients had high EBV titers. Detection of viral genomes in the neoplastic cells of BL and NPC has confirmed the postulated association.

The possible role of EBV in Hodgkin's disease (HD) was more ambiguous. HD patients were more frequently seropositive than matched controls and their mean antibody titers were higher, but a substantial minority of the patients was seronegative. The titer elevations were therefore written off as probable secondary consequences of the HD-associated immune defects. Antiviral (EA and VCA) titers were known to increase in T-cell deficiencies.

The detection of EBV genomes in HD cells brought EBV again to the forefront of interest after a dormancy of 2 decades. Improved techniques of in situ hybridization and particularly the recent use of probes for the highly transcribed EBV-encoded small RNAs (EBERs) present at a level of about 1 million molecules in most EBV-carrying cells opened the field for the detection and identification of virally infected cells in complex tissues. Using a highly sensitive single-stranded EBER antisense probe, Herbst et al.1 now show in a report in this issue of Blood that virtually all neoplastic cells of EBV-DNA carrying HD cases are EBER positive. Their finding that only about half of the HD tumors carry EBV genomes is in line with earlier studies reviewed in this report.

Mutatis mutandis, the situation is not entirely unlike the well known relationship between EBV and BL. About 97% of the high endemic African BLs carry viral DNA. However, only one-fifth of the nonendemic (sporadic) BLs are EBV positive. Acquired immunodeficiency virus (AIDS)-associated BLs carry EBV in about 40%. Whether EBV negative or positive, all BLs carry an Ig/myc translocation. The latter is therefore regarded as the unifying event in BL. The regular association of EBV with the high endemic form has been taken to indicate, nevertheless, that the virus must play some role in the etiology of the tumor, perhaps by expanding and/or immortalizing a subset of Ig/myc translocation-prone B cells.

As reviewed by Herbst et al.,2 mixed cellularity (MC) and nodular sclerosis (NS) are the most frequent EBV-positive forms of HD, in that order. Lymphocyte predominance (LP) forms are largely negative. This is in line with earlier serologic findings.3

The detection of EBV-DNA in HD tissues has provoked much discussion. Are the viral genomes harbored by the neoplastic cells? If so, are all of them positive in a given tumor or only some? Are they also present in reactive normal cells?

Similar questions have been raised during the early days of NPC research after the association of NPC with EBV had first been discovered.4 This carcinoma is often heavily infiltrated with small lymphocytes. Because there was no previous evidence to show that EBV could infect epithelial cells, it was first naturally assumed that the viral genomes must reside in the lymphocytes. This was readily falsified by the demonstration that the carcinoma cells carried multiple viral genomes and expressed EBV nuclear antigen (EBNA). The lymphocytes were largely T cells. Their presence is still enigmatic, because they are phenotypically resting rather than activated T cells. They do not carry EBV.

In HD, the variable appearance and the complexity of the neoplastic lesion, the approximately 50% overall association with the virus, and the differences in the positivity of the morphologic subtypes create further problems. The immunohistochemical distinction of Herbst et al.2 between EBV-carrying HD cells and normal lymphocytes is an important improvement. In contrast to the uniform EBER

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expression of all neoplastic cells in the EBV-harboring HD cases, only a small number of normal B cells were positive. They were also present in EBV-negative HD lesions. These distinctions have brought the possible role of the virus in the EBV-positive HD lesions into a much sharper focus. That does not mean that the relationship has been explained.

Many questions remain enigmatic. If the neoplastic HD cells belong to the B-cell series, why does their known viral antigen expression (EBNA-2, LMP) resemble the epithelial cells of NPC, rather than BL cells (EBNA-1 only) or the EBV-carrying immunoblastomas that arise in immunodefectives (EBNA-1 through -6 and LMP)? Recent studies on cell phenotype-dependent differences in viral genome expression indicate that the virus uses differentiation-dependent cellular factors to regulate its own nonlytic, growth transformation-associated protein synthesis. Phenotypically, BL cells resemble resting B cells rather than immunoblasts, but can apparently not come to a rest, due to the onward driving force of the translocation activated myc gene.

It is interesting in this connection that the small EBER-positive B lymphocytes that Herbst et al have found in both EBV-carrying and EBV-negative HD tissues failed to express LMP. This is consistent with the hypothesis that the viral antigen expression of normal resting B cells may resemble BL cells. The neoplastic cells in HD differ from them and also from the fully expressing EBV-transformed immunoblasts of normal B-cell origin, as already mentioned. Are we to envisage a third type of viral regulation in the putative normal lymphoid precursors of HD? Can normal cells with a corresponding viral expression provide interesting clues in the search for still undefined HD precursor(s)?

Is there any relationship between infectious mononucleosis (IM) and HD? Some relationship has been postulated on the basis of certain epidemiologic similarities, particularly the preferential appearance of both diseases in high socioeconomic groups, and the documented appearance of HD in the wake of IM in a number of cases. Does the delayed EBV infection of hygienically protected subpopulations increase the risk of HD in a similar way as it increases the likelihood of clinically manifested mononucleosis, or is this relatively slight epidemiologic similarity of the two diseases purely coincidental, perhaps related to the EBV-like sociology of some unknown infectious agent in HD? Separate epidemiologic studies on EBV-carrying compared with EBV-negative cases of HD would be of great interest in this context.

HD patients often have a strongly impaired T-cell immunity. Nevertheless, the EBV-carrying immunoblastomas that appear in transplant recipients and some congenital immunodeficiencies have not been found in HD patients. What is the difference between these immune defects? What effectors prevent the immunoblastomas from appearing in severely T-cell-impaired HD patients?

The study of EBV-specific T-cell responses in HD will probably start by analyzing infiltrating T cells in the HD lesions. Have they been sensitized to EBV antigens expressed by the neoplastic cells? If not, why not? If they have, why are the neoplastic cells not rejected?

Immune surveillance against EBV is nearly watertight in normal seropositives. This is the reason why mononucleosis is a self-limiting disease. EBV-transformed immunoblasts express at least eight proteins. Several of them, notably EBNA-2, 3, 4, and -6, and LMP1 and LMP2, have been shown to generate CTL recognizable targets, probably by accidental fitting of their processed peptide derivates to the groove of appropriate major histocompatibility complex (MHC) class I molecules. The HLA polymorphism of our species is apparently sufficient to provide enough CTL targets for the good protection that we enjoy. Alternatively, or in addition, non-T effectors may play a role as well.

The immunoblastomas of the immunodefectives express all potentially immunogenic viral products. They can only grow in immunodefective hosts. BL cells escape recognition, partly due to the downregulation of their potentially immunogenic proteins (except EBNA-1, which may be less or non immunogenic for T cells) and partly because of their phenotypically determined low expression of adhesion molecules and some HLA class I antigens. In view of the fact that the potentially highly immunogenic LMP1 protein is expressed in HD cells, reasons will have to be found for the absence of rejection. Considerable LMP1 sequence variations are known to occur between different viral substrains. Is it possible that less immunogenic forms are expressed in HD cells? Alternatively, could the HD cell phenotype resist rejection for its own phenotypic reasons, in analogy with the BL cell?

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