Breast Cancer: a New Epstein-Barr Virus-Associated Disease?

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Epstein-Barr virus (EBV) has learned, remarkably successfully, to live with its human host. However, this ability was not acquired overnight. Closely related viruses infect old-world primates, including our closest relatives, the gorilla and chimpanzee (1), suggesting that EBV, or its immediate ancestors, has been with us since long before we were human. This association over millions of years has led to such intimacy that at least 90% of the world’s adult population, including people in the most remote corners of the world, are infected by the virus, the vast majority with no serious consequences. Even infectious mononucleosis, a largely benign illness that frequently accompanies primary infection in adolescence or early adulthood, is likely to have been rare until well into the industrial era, since clinically silent infection with EBV in infancy is the norm in developing countries. However, even the best of marriages have their ups and downs, and EBV has been impugned in the causation of more serious illnesses, including malignant neoplasms, albeit in a tiny fraction of the population. Indeed, EBV was discovered because of its presence in African Burkitt’s lymphoma and has been shown in the ensuing 35 years or so to be associated with a growing list of neoplasms—particularly, but not exclusively, tumors of lymphoid or epithelial origin (2). The latest, if rather controversial, addition to the list is breast cancer. In this issue of the Journal, Bonnet et al. (3) provide data in support of earlier reports demonstrating the presence of EBV in breast cancer (4,5). At least five other groups (6–10), however, have reported negative results. This is an important issue that needs to be resolved, for an association of EBV with breast cancer would have potential relevance to its early detection, treatment, and even prevention.

Why the controversy? The association of EBV with lymphoid tumors of B-cell origin—such as Burkitt’s lymphoma—is not greatly surprising, since once EBV gains entrance to a new host, via salivary transmission, it infects B lymphocytes in the oropharynx and continues to reside in B cells throughout the life of the individual. Although present in some T-cell lymphomas, its relationship with normal T cells is less clear. The scenario with respect to epithelial tumors is still evolving. For many years, it was believed that infectious virus in saliva was generated from infected epithelial cells in the nasopharynx (11), which provided a convenient explanation for its variable presence in the epithelial cell component of lymphoepithelioma or nasopharyngeal carcinoma (NPC), the second malignant neoplasm to be shown to be associated with EBV. The high prevalence of NPC in certain populations, such as southern Chinese, is probably accounted for by local cofactors, one being the weaning of infants on salted fish, although a genetic predisposition has also been implicated (12). Recently, however, the inability to detect EBV by in situ hybridization (ISH) with probes for EBER (small EBV RNA molecules) in epithelium overlying tonsils removed at surgery has shed doubt on the long-held belief that the source of virus in saliva is nasopharyngeal epithelium (13). This finding has relevance to the viral life cycle, to the potential role of EBV in epithelial cancers, and to the apparently contradictory results in the specific context of breast cancer.

First, there is the issue of EBV detection. Bonnet et al. (3) used several methods, including polymerase chain reaction (PCR) assays, Southern blotting, and immunohistochemistry with monoclonal antibodies against EBNA-1, a viral protein required for EBV to persist in cells and which is invariably expressed in tumors associated with EBV. Each of these methods gave positive results, although Southern blotting and immunohistochemistry were performed on smaller subsets of the PCR-positive samples. While PCR can detect EBV in normal lymphocytes and by itself is not proof of the presence of EBV in tumor cells, Bonnet et al. were unable to detect viral sequences by PCR in the majority of normal tissue samples taken from areas adjacent to the tumor. Of interest, EBER ISH, which is widely used, and generally a very sensitive method for detecting the presence of EBV because of the high EBER RNA copy number, was negative in the hands of these investigators, although they tested only four of the PCR-positive samples. The absence of EBER expression is intriguing; in NPC, absent EBER expression has been reported in cases positive for EBV by in situ PCR (14). Labrecque et al. (4), in their report of a positive association of EBV with breast cancer, noted that EBER ISH was positive in only half as many PCR-positive tumors as was DNA–DNA ISH, a form of ISH in which both target and probe are DNA (4). The possibility that there is variable or absent expression of EBER in EBV-positive breast cancer cells may explain, at least in part, the apparently conflicting results in the literature. Two of the groups that reported a lack of association of EBV with breast cancer of various histologies used only EBER ISH to detect EBV (6,7). A third, in which only three cases were studied, obtained positive results by PCR and negative results by EBER ISH (10). The remaining two, which used several techniques to detect EBV, examined only medullary carcinoma of the breast (8,9), a rare tumor (there was one in the series by Bonnet et al.) that is histologically similar to lymphoepithelioma-like carcinoma, a group of tumors that is often, but not always, EBV associated (15). Thus, although more data are needed, it seems likely at this time that EBV is frequently associated with multiple histologic types of breast cancer. The variable expression of EBER in neoplastic epithelial cells also leaves open the possibility that EBV may be associated with a broader range of tumors than previously thought, because many...
investigators in recent years have relied upon EBER ISH as a means of assessing EBV association. EBER ISH, if positive, is meaningful; however, if it is negative, it does not prove the absence of EBV, particularly in epithelial cells.

Second, there is the question of how, or even whether, EBV enters normal epithelial cells, a point that is relevant to its potential oncogenic role in epithelial neoplasms. As noted earlier, the inability to detect EBER in normal pharyngeal epithelium provided grounds to question the notion that pharyngeal epithelium provides the source of salivary virus (13). Even with other techniques (e.g., DNA–DNA ISH), EBV has generally not been detected in the normal epithelium adjacent to tumors of the upper aerodigestive tract, but epithelium over tonsillar lymphoid tissue has recently been shown to contain high copy numbers of EBV (although without EBER expression), suggesting that epithelium overlying lymphoid tissue may be a special case and that the standard version of virus transmission cannot yet be discarded (16). It is also possible that EBV can more readily infect or persist in abnormal epithelial cells—indeed, a variety of preneoplastic or dysplastic epithelial lesions have been shown to contain EBV (17,18). Thus, EBV may be of pathogenetic relevance only in the presence of environmental carcinogens or pre-existing epithelial damage, a possibility entirely consistent with the epidemiology of NPC. One interpretation of the available data is that EBV permanently resides in lymphocytes from where it may passage to epithelial cells (including those in the breast). The virus enters a lytic phase as the cells differentiate, but dysplasia may prevent this, providing an opportunity for EBV to contribute to carcinogenesis.

The final question to be addressed is whether EBV, if we accept its association with breast cancer, plays a causal role or, alternatively, may sometimes infect pre-existing tumor cells. The evidence from immunohistochemistry suggests that EBV is not present in 100% of the malignant cells (3), thus arguing that it was absent from the neoplastic clone at the time of malignant transformation, although selective loss of virus would remain a formal possibility. Whether or not EBV is of pathogenic significance in breast cancer, it could still (as suggested by the data of Bonnet et al.) be a useful prognostic indicator or even provide a molecular target for therapy (19). If, however, the virus were shown to be an etiologic factor in a substantial proportion of breast cancers, there would be strong grounds for speeding up efforts to develop an EBV vaccine, since breast cancer is the most common tumor of women worldwide, with approximately 800,000 new cases per year (20).

REFERENCES