Learning Objectives:

1. Review the biology of EBV infection
2. Discuss the different EBV-associated neoplasms
3. Discuss the role EBV may play in pathogenesis

Epstein-Barr virus is a human herpesvirus (along with herpes simplex 1 and 2, CMV, varicella zoster virus, HHV-6, HHV-7, and Kaposi sarcoma herpesvirus). Mostly closely related to KSHV, it is the only gamma-1 herpesvirus. It has a large genome, 171 kbp in length, and has been entirely cloned and sequenced. It is a linear double-stranded length of DNA with non-random single-stranded breaks but may circularize to form episome within human cells. Its genome has five unique regions of DNA, punctuated by four internal repeat regions and capped on either end by a terminal repeat region.

Virtually all adults are infected with EBV, and the infection persists for life. In underdeveloped countries, the infection occurs in early childhood, usually without symptoms; while in developed countries of high socioeconomic status the infection occurs most often in teens and young adults, one-third of cases manifesting clinically as acute infectious mononucleosis. In developed countries of low socioeconomic status infection generally occurs in an intermediate age group, generally by the age of 15.

In the initial infection, an active (lytic) infection is seen. In a lytic infection, the virus is in a linear configuration. There is expression of the immediate early genes, including BZLF1 and BZRF1 encoding the Z and R proteins, respectively, responsible for viral replication. Late genes are then transcribed, resulting in the production of the structural components found in free virions (nucleocapsid proteins, membrane-associated glycoproteins, and the viral homologues BCRF1 (IL-10) and BHRF1 (BCL2). It is the lytic infection that is inhibited by acyclovir and gancyclovir. In a latent infection, which occurs soon after the lytic phase, the virus persists as 1-100 episomes; there may be some integration into the human genome. Only a small portion of the viral genome is expressed including EBNA-1, 2, 3A-C, and LP; LMP-1, 2A, and 2B; and EBER-1 and 2, BARF-0 and 1, and BHRF-1. In addition, there is an upregulation of a variety of host genes, including adhesion antigens (LFA-1, LFA-3, and ICAM-1), LP enhances the ability of EBNA-2 to up-regulate LMP1. LMP-1 is a transforming agent, and can act as an oncogen in transfectant systems. It induces host adhesion and activation antigens and bcl-2. It is a homologue of the TNF receptor superfamily, and can take the place of TNF in activating TRAF1 and 2 and ultimately activating NF-kappaB. LMP-2A blocks the switch from latent infection to lytic infection. Its expression in transgenic mice allows non-transformed Ig-negative B-cells to colonize peripheral lymphoid organs. BARF-1 is a CSF receptor homologue. It also has malignant transforming activity as well as an immortalizing effect on human epithelial cells in vitro. The function of EBER-1 and 2 is still not clear but each is amplified up to $10^5$ to $10^6$ times, making them excellent targets for in situ hybridization studies.

EBV generally infects individuals by spread via saliva. It is not certain what role the surface epithelium plays, but infection of B-cells occurs soon after entrance of the virus through the mucosa, with EBV
entering the cells via the CD21 (CR2) receptor. In the absence of prior immunity, a massive infection ensues (up to 1:10 B-lymphocytes) and the full program of latent genes is expressed (latency pattern 3). Soon, however, viral latency antigens are presented on MHC class 1, and a cytotoxic CD8\(^+\) T-cell response occurs within weeks. Eventually, a resting EBV infection occurs (predominantly in memory B-cells), within either minimal (EBNA-1 only) (latency pattern 1) or no EBV latent antigen expression. At this point, only about 1 in a million B-cells in the peripheral blood and between a thousand and ten-thousand cells in peripheral lymphoid organs are thought to carry the infection. In a subset of infected cells, under the right conditions, there is a switch to a lytic cycle (thought to occur mainly in plasma cells). Cell lysis occurs with release of virions, some of which enter saliva, and the cycle repeats itself.

**EBV and Classical Hodgkin Lymphoma**

For many years, there had been compelling epidemiologic data suggesting a relationship between EBV and Hodgkin lymphoma (HL). Patients with a history of infectious mononucleosis have a 2-4 times incidence of HL, particularly EBV-associated HL, with a mean interval of 4 years. The proportion of patients with HL who have higher titers of antibody against EBV capsid antigen is higher than expected by change, and importantly, patients with HL have elevated IgG and IgA against EBV capsid antigen months to years prior to the diagnosis of HL. EBV may be identified in 20-40\% of cases of classical HL by Southern blot hybridization, and analysis of the configuration of the terminal repeat regions reveals monoclonal episomes, identical at all sites, indicating the presence of EBV in a clonal population of cells. EBV is amplified at least 50-fold in the EBV+ cells. PCR reveals EBV in 50-90\% of the cells, but cannot distinguish EBV in Hodgkin cells from EBV in bystander B and T-lymphocytes. However, these studies have demonstrated that the EBV is strain A in most cases and strain A or B in HIV-associated cases. EBV is identified in 20-30\% of cases using DNA-DNA in situ hybridization but 30-50\% of cases using DNA/RNA-RNA in situ hybridization studies using EBER probes. In a given positive case, all or virtually all the Hodgkin cells are EBV positive. If one site is positive, all sites are positive. Rare small lymphocytes may also be positive. These probably represent EBV-positive bystander B-lymphocytes, but the possibility of Hodgkin “stem” cells cannot be completely ruled out. By immunohistochemical studies, Hodgkin cells have a latency pattern 2, with expression of EBV LMP-1 and 2 and EBNA-1. LMP-1 is a consistent marker for EBV-positive Hodgkin cells, but is negative in the EBV-positive small lymphocytes. The Hodgkin cells are negative for EBNA-2 and lytic proteins, although a minority of EBV-positive Hodgkin cells may express BZLF1, suggesting an abortive lytic infection.

EBV is positive in 30-50\% of HL cases in Western populations, but has a higher incidence in other population, being 50-95\% positive in Central and South America, 95\% in cases from equatorial Africa, and 65\% positive in cases from China and Japan. EBV is positive in 80\% of cases of mixed cellularity and lymphocyte depletion, 25\% of cases of nodular sclerosis, but only rare cases of nodular lymphocyte predominance. There is an increased incidence in patients over the age of 50 or under the age of 15 years. There is no correlation shown so far with specific HLA groups, although familial cases usually have a negative correlation with EBV. HIV-associated HL is almost 100\% EBV-positive. In stage I, there is an association with presentation in neck lymph nodes, although there is no overall correlation with stage. There is no correlation with a B-cell phenotype. There is an inverse correlation with inactivation of the A20 protein encoded by the TNFAIP3 gene, suggesting a role for EBV in the pathogenesis of EBV-associated HL.

**EBV and B-Cell Lymphoma**

There are several experimental models of EBV-associated B-cell lymphoma. SCID mice reconstituted with cells from EBV+ donors develop lymphoproliferative disorders, which may be prevented by anti-CD20 or anti-CD40 antibodies. In addition EBV forms lymphomas in cottontop tamarins (a new-World monkey) that resembles post-transplantation lymphoproliferative disorders. B-cell lymphoma associated with EBV include Burkitt lymphoma, lymphomas associated with immunodeficiency (including post-transplantation, HIV-associated, congenital, methotrexate/steroid treatment for autoimmune diseases, and aging-associated). In addition, diffuse large B-cell lymphoma associated with chronic inflammation and
lymphomatoid granulomatosis are EBV-associated. Hairy cells leukemia and central nervous system lymphomas not associated with immunosuppression are NOT EBV-associated (in contrast to what was one-time believed). The association between EBV and Burkitt lymphoma is >95% in endemic cases, 10-30% in sporadic cases and 30-50% in AIDS-associated cases. In all EBV-associated cases, EBV is in all or virtually all the neoplastic cells. There is almost always a latency pattern 1, with EBNA-1 but not LMP-1 expression. There is some evidence that EBV-BL arises from a latency pattern-3 progenitor, and that the principal selection pressure is for down-regulation of the c-myc antagonist EBNA-2. EBNA-1 expression is critical for survival of EBV-associated BL. Immunoglobulin gene analysis has revealed that EBV-associated cases have higher mutation rates and signs of antigen selection in comparison to EBV-negative cases. It has been shown that B-cell differentiation in EBV-associated BL is impaired at the post-transcriptional level by a change of miRNA-altered expression, preventing exit from the germinal center. This may also provide a mechanism for the pathogenesis of Burkitt lymphoma in the rare c-myc negative cases.

EBV may be seen in both benign and malignant lymphoid proliferations in HIV-infected patients. The numbers of EBER-positive cells is increased over normal in HIV-associated lymphadenopathy, and increases as a patient progresses from reactive follicular hyperplasia to lymphocyte depletion. The EBV-positive cells are usually present in the paracortical areas, but occasional germinal centers may have many EBER+ cells, suggesting a “miniclonal” proliferation. HIV-associated lymphomas that are associated with EBV include systemic diffuse large B-cell lymphoma (20% EBV+), Burkitt lymphoma (30-50% EBV+), diffuse large B-cell lymphoma of the CNS (100% EBV+), plasmablastic (60-70% EBV+), primary effusion lymphoma (80% EBV+ in addition to 100% KSHV+), polymorphic proliferations (usually EBV+) and classical HL (100% EBV+).

Post-transplantation lymphoproliferative disorders (PTLD) have been well-known to be EBV positive. Actually, today only 80% of cases are EBV+, showing a variable but usually type 3 latency program. EBV-seronegative cases at transplant are more likely to develop PTLD. In early benign PTLDs, EBER shows scattered positive cells. In polymorphic lesions, many EBV+ cells are seen, and the EBV is usually in a clonal population of cells as determined by terminal repeat analysis (although there may be multiple clones). In monomorphic lesions and in Hodgkin lymphoma, the EBV is present in all or virtually all the neoplastic cells. The rare peripheral T-cell lymphomas arising in this setting are EBV+ in about 50% of cases.

Lymphoproliferative disorders have also been reported in patients with rheumatoid arthritis (80%), dermatomyositis, or psoriasis treated with methotrexate, TNF blocks, and other immunosuppressive medicines. Most cases look like diffuse large B-cell lymphoma, although some are Hodgkin lymphoma/Hodgkin-like lesions or polymorphic proliferations. About 50% of these cases are EBV+. They may regress following cessation of treatment, particularly the HL-like and polymorphic lesions and those cases associated with EBV.

EBV+ diffuse large B-cell lymphoma of the elderly is a lymphoma occurring in elderly patients with no other cause of immunodeficiency. It occurs with increasing incidence with increasing age, and includes up to 10% of elderly lymphoma in Asian populations. There is a slight male predominance. Most patients present with extranodal disease, including skin, lung, tonsil, and stomach. There is usually an intermediate to high International Prognostic Index and the disease is aggressive with a poor prognosis, which tracks with age. A diffuse architecture is seen, often with necrosis. There is either a polymorphous appearance, with a range of B-cell maturation which may include R-S-like cells, or a monomorphic appearance, with either centroblastic or immunoblastic features. It is a B-cell lymphoma, usually with an activated non-germline center phenotype. EBER is positive in the neoplastic cells, and LMP is usually but not always positive. Terminal repeat studies show EBV present in a clonal population of cells.

Diffuse large B-cell lymphoma associated with chronic inflammation is a neoplasm of large transformed B-cells arising in the context of local chronic inflammation. It usually arises decades after exposure to the inflammation; therefore, most patients are quite elderly, with a male predominance. Most cases have
been reported in association with pyothorax occurring many years after induced pneumothorax for pulmonary tuberculosis, although bone (chronic osteomyelitis), joint, and soft tissues are other common sites. Although complete resection may be a treatment option because these lymphomas sometimes stay localized, it is usually an aggressive lymphoma, with a 25% five-year survival. The histologic appearance is similar to EBV+ diffuse large cell lymphoma of the elderly. It is a B-cell neoplasm, with an activated non-germinal center phenotype. EBER is consistently positive, and LMP-1 is usually but not always positive.

Lymphomatoid granulomatosis is an angiocentric and angiodestructive lymphoproliferative disease involving extranodal sites, composed of EBV+ B-cells admixed with reactive T-cells, which usually predominate. Patients often have an underlying immunodeficiency, including post-transplantation therapy, Wiskott-Aldrich syndrome, or X-linked lymphoproliferative syndrome. Extranodal organs are almost exclusively involved, including the lungs (90%), skin (25-50%), kidney (33%), brain (25%), and liver (25%). There are scattered large cells positive for B-lineage antigens, in a background of numerous small T-cells. The large cells are EBER+, CD30 +/-, EBV-LMP +/- and CD15-. Cases with greater numbers of large cells (grade II and III) usually show clonal immunoglobulin gene rearrangements.

EBV and T Cell Lymphoma

T-cell lymphomas specifically associated with EBV include extranodal NK/T cell lymphoma, nasal type, systemic EBV+ T cell lymphoma, hydroa vacciniforme lymphoma, and angioimmunoblastic T-cell lymphoma. However, almost any T-cell lymphoma may be T-cell associated in a minority of cases, particularly in Asian populations, and many T-cell lymphomas may contain scattered EBV+ cells, a reflection of the generalized immunodeficiency that is sometimes part of a peripheral T-cell lymphoma.

Extranodal NK/T-cell lymphoma, nasal type, is a distinctive predominantly extranodal lymphoma characterized by vascular damage and association with EBV, of mostly NK cytotoxic phenotype. It is much more common in Asians and the native American population of Central and South America. There is a strong association with EBV, regardless of ethnicity, and this neoplasm is perhaps the most consistently EBV-associated malignant lymphoma. EBER is almost always positive, however, LMP-1 is usually negative.

Systemic EBV+ T-cell lymphoma is a recently recognized clonal proliferation of EBV-infected T-cells with an activated cytotoxic phenotype. It occurs primarily in children and young adults. It can occur shortly after primary acute EBV infection or in the setting of chronic EBV infection (over 6 months). It is a systemic disease, commonly involving the liver, spleen, lymph nodes, bone marrow, skin, and the lungs. There is usually a rapid progression, with multi-organ failure leading to sepsis and death, with a clinical course lasting from days to weeks. Pathologically, a sinusoidal pattern of infiltration is usually seen. Despite the aggressive clinical course, most cases lack striking cytologic atypia, although some cases may feature pleomorphic cells. There may be prominent hemophagocytosis, and it may mimic lymphohistiocytic hemophagocytosis. There is a T-cell phenotype, lacking CD56. TIA-1 is positive. There are clonal TCR gene rearrangements. EBER is consistently positive, and terminal repeat analysis demonstrates that the EBV is in a clonal population of cells.

Hydroa vacciniforme lymphoma is a recently described cutaneous T-cell lymphoma. It also occurs primarily in children, mainly Asians, native Americans, or central South Americans. It has an interesting association with a sensitivity to insect bites and the sun, and it usually occurs on sun-exposed skin such as the face. Patients develop a papulovesicular eruption that ulcerates, although there may also be systemic symptoms. The clinical course is variable, and it may behave indolently for many years. It is thought to be due to a defective cytotoxic response to EBV. Pathologically, once sees often quite extensive infiltrates, extending from the epidermis to the subcutis, often with necrosis, angiocentricity, and angioinvasion. There is a proliferation of small- to medium-sized lymphoid cells with a great deal of atypia. Most, but not all cases have clonal TCR gene rearrangements. It consistently EBER+ with EBV present in a clonal population of cells.
Cases of angioimmunoblastic T-cell lymphoma (AITL) are also associated with EBV in a high proportion of cases, but in this lymphoma, the pattern of EBV is distinctly different. In contrast to all the other lymphomas discussed, the EBV in AITL is primarily in the B-cells, usually scattered small and large cells. Thus it is probably not part of the pathogenesis of the disease, but a manifestation of the diminished local immunity. However, the EBV+ B-cells may contribute to the formation of B-clones in this lymphoma, and the occasional progression to an EBV+ B-cell lymphoma.

EBV and Epithelial Neoplasms

There is a significant association of EBV in nasopharyngeal carcinoma, gastric carcinoma, as well as other foregut-type lymphoepitheliomas. It is still not entirely clear how EBV enters epithelial cells, although the latest evidence suggests that it is likely to be mediated between EBV gHgL surface glycoproteins and the integrins \( \alpha v 6 \) and \( \alpha v 8 \). There is a near 100% association in undifferentiated/non-keratinizing subtypes of nasopharyngeal carcinoma, as well as a weaker association with keratinizing subtypes. The data on the latter is interesting, as a near 100% association is seen in populations from Southern China, with a less than 50% association seen in Western populations. Cases of EBV-positive nasopharyngeal carcinoma are particularly common in Southern China, possibly related to certain HLA antigen profiles and/or environmental factors (e.g., consumption of slated fish, etc.). EBV is also identified in precursor lesion, particularly carcinoma in-situ. A type I or II latency pattern is seen.

EBV is associated with gastric carcinoma in about 10% of cases. It is seen in virtually all cases with a lymphoepithelioma-like or lymphoid stroma-rich histology, but only about 5-10% of cases with typical histology. It is more common in those cases with a “lace”-like histology. EBV-associated cases are more frequently seen in males than females, and is more commonly seen in younger patients. It is more common in tumors occurring in the proximal stomach and particularly gastric-remnant tumors. It is more common in tumors of lower stage (less lymph node involvement), and stage-for-stage has a better prognosis. The EBV is present in the tumor cells, as well adjacent dysplastic foci. A latency pattern 1 is typically seen, with the exception that LMP2A is also usually expressed. Aberrant DNA methylation may be a primary molecular lesion, mediated via LMP2A.

A wide variety of foregut-origin lymphoepithelial carcinoma may be EBV-associated, including neoplasms from the salivary glands (particularly common in eskimos and termed eskimomas by some), thymus, and lung.

EBV and Mesenchymal Neoplasms

EBV has been identified in the neoplastic cells in leiomyosarcomas occurring in HIV-infected patients, as well as a subset of inflammatory pseudotumor-like follicular dendritic cell tumors occurring in the liver and spleen.

EBV and Neoplasms: Conclusions

EBV infections B-cells and, to a lesser extent, T-cells and is associated with Hodgkin lymphoma and a variety of B- and T-cell lymphomas. EBV also infects epithelial cells and has a significant association with nasopharyngeal, and to a lesser extent, gastric carcinoma. Finally, EBV is also associated with rare mesenchymal neoplasms. The role that EBV plays in the pathogenesis of any of these neoplasms is still not clear, although progress is being made in several lymphomas, including Hodgkin lymphoma and Burkitt lymphoma. Regardless of the role in pathogenesis, EBV represents a tool for diagnosis and EBV antigens may represent a target for future therapeutic interventions. Finally, the development of EBV vaccines may have a significant impact on lymphoma prevention.
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