Epstein-Barr Virus Hepatitis
A Review of Clinicopathologic Features and Differential Diagnosis
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Epstein-Barr virus (EBV), a member of the herpesvirus family, infects oropharyngeal epithelial cells and B cells, resulting in an expansion of cytotoxic CD8 T cells and consequent atypical lymphocytosis. Approximately 90% to 95% of the population worldwide is seropositive. Primary EBV infection is common in young children and is frequently asymptomatic. Symptomatic EBV infection typically presents in adolescents, with tonsillitis, cervical lymphadenopathy, and fever characteristic of infectious mononucleosis. Typical presenting signs and symptoms of infectious mononucleosis may be absent, however, confusing the diagnosis. Although approximately one-half of patients with infectious mononucleosis have splenomegaly, only about 14% are reported to have hepatomegaly. In more than 90% of cases of EBV-related mononucleosis, liver involvement is present, but it is often either subclinical and self-limited or manageable with supportive treatment alone. Generally, it manifests as transaminase elevations that are transient and mild (2–3 times the upper limit of normal); infrequently, patients have more striking transaminase elevations (5–10 times the upper limit of normal), with jaundice reported in a small minority (less than 5%) of patients. Systemic symptoms of mononucleosis are typically present when EBV involves the liver; however, hepatic involvement can also occur in isolation. Severe or fatal hepatitis from EBV infection has also been observed, with rare instances reported in immunocompetent patients and more frequent occurrence in immunocompromised individuals. Rarely, there can be recurrent or chronic infections lasting longer than 6 months, which is termed chronic active Epstein-Barr virus infection and is associated with a very poor prognosis. Hepatic findings vary but hepatic failure is a significant contributor to high mortality rates.

Conclusions.—Characteristic microscopic findings in EBV hepatitis include a diffuse lymphocytic sinusoidal infiltrate in a “string of beads” pattern, expansion of portal tracts by a predominantly lymphocytic infiltrate, and intact lobular architecture. In situ hybridization of EBV-encoded RNA is a helpful ancillary test. Correlation of clinical history, laboratory findings, and histopathologic features is essential to distinguish EBV hepatitis from autoimmune liver diseases, transplant rejection, lymphomas, and drug-induced liver injury.

Laboratory Findings
The diagnosis of EBV hepatitis is usually made based on clinical presentation in combination with laboratory findings. Lymphocytosis with numerous atypical lymphocytes and elevated liver enzymes are most often observed in conjunction with the detection of EBV-specific serologies or heterophile antibodies. Heterophile antibodies are typically associated with EBV in adolescents and adults; however, they also cross-react with antigens found on cells of other animal species. In addition, they have been reported in autoimmune diseases, HIV infection, lymphomas, and other viral infections. Heterophile antibodies also have a significant rate of false-negative results; moreover, in children with EBV mononucleosis, only a few will have detectable heterophile antibodies. Thus, heterophile antibodies are limited in specificity as well as in sensitivity.

At the onset of symptoms, most patients with primary EBV infections will have detectable immunoglobulin (Ig) M antibodies to EBV viral capsid antigen (VCA), which typically disappear within 4 to 6 weeks. IgG antibodies to VCA first appear in the initial month of illness, whereas IgG antibodies against Epstein-Barr nuclear antigen 1
EBNA-1 typically appear in the convalescence stage. Both anti-VCA IgG and anti–EBNA-1 IgG persist for life. Thus, primary infection is indicated by the presence of anti-VCA IgM and absence of anti–EBNA-1 IgG, with or without anti-VCA IgG. Conversely, primary infection is highly unlikely if anti–EBNA-1 IgG and anti-VCA IgG are present, but IgM is absent. These serologic studies may also be unreliable in young children and in immunocompromised patients with incomplete humoral responses.

Use of quantitative polymerase chain reaction (PCR) to determine EBV viral load is typically reserved for immunocompromised patients and patients at risk for developing posttransplant lymphoproliferative disorder. Autoimmune antibody production, such as antinuclear antibodies or anti–smooth muscle antibodies, can also be associated with EBV, perhaps through cross-reactivity between EBV proteins and autoantigens.

(HISTOPATHOLOGY)

Epstein-Barr virus hepatitis is typically diagnosed clinically; however, a liver biopsy may be indicated if the clinical presentation is atypical or laboratory findings are ambiguous or unusual. A range of histopathologic features can be observed in EBV hepatitis, but the most characteristic histologic feature is a diffuse lymphocytic sinusoidal infiltrate in a single file or “string of beads” pattern. Commonly, the portal tracts are expanded by an infiltrate that is predominantly lymphocytic but can also include occasional admixed plasma cells, neutrophils, and eosinophils. The lymphocytic component of the infiltrate consists primarily of reactive cytotoxic (CD8) T lymphocytes, and intermixed natural killer cells, with infrequent EBV-infected B cells. Large atypical lymphocytes are often present. The overall lobular architecture remains predominantly intact, although hepatocytes may show mild swelling, vacuolation, and steatosis with focal apoptosis (Figure 1, A through D). Confluent necrosis and cholestatic hepatitis are rare.

(Ancillary studies)

Ancillary techniques for histopathologic diagnosis include PCR and in situ hybridization staining of EBV-encoded RNA (EBER). These methods have equivalent sensitivity for detecting EBV in formalin-fixed, paraffin-embedded speci-
mens of native livers from immunocompetent patients. Scattered EBER positivity in lymphocytes should be interpreted as significant because EBER stains EBV-infected B cells that account for a few of the inflammatory infiltrates (Figure 3). It is important to note that EBV-associated hepatitis is not excluded by a negative EBER, especially in the setting of a small biopsy because staining may be focal. Immunohistochemical staining for EBV latent membrane proteins is not useful in establishing a diagnosis; it is typically absent in immunocompetent patients, whereas in patients with posttransplant lymphoproliferative disorder, latent membrane protein staining may be present but is a less-sensitive test than PCR or EBER.

**DIFFERENTIAL DIAGNOSIS**

The histopathologic changes induced in the liver by EBV are characteristic of, but not specific for, EBV hepatitis. The differential diagnosis includes autoimmune liver diseases, transplant rejection, lymphomas, and drug-induced liver injury. Distinguishing between these entities may not be possible with histologic findings alone, and correlation with clinical and laboratory data is usually necessary to establish the correct diagnosis.
**Autoimmune Hepatitis**

As noted, autoimmune antibody production has been associated with EBV, and the presence of positive autoimmune antibodies and elevated serum transaminase levels will often raise the possibility of autoimmune liver disease. The presence of positive EBV serologies, elevated levels of IgG, and the presence of other autoantibodies (anti-smooth muscle cell antibodies or liver–kidney–microsomal antibodies) can help distinguish EBV hepatitis from autoimmune hepatitis. In addition, autoimmune hepatitis typically has a more striking plasmacytosis as well as more significant lobular activity and hepatocellular damage, including bridging necrosis. In contrast, EBV hepatitis lacks prominent plasma cells and the lobular architecture is generally intact, with only spotty necrosis of hepatocytes.

**Transplant Rejection**

Immunosuppressive therapy reduces the risk of liver transplant rejection but predisposes liver transplant recipients to latent EBV reactivation and increases the possibility of clinically significant primary EBV infection. When EBV hepatitis does occur in a liver transplant recipient, it may be difficult to distinguish from acute cellular rejection on histologic grounds, particularly as EBV hepatitis can cause endothelial inflammation. In EBV hepatitis, the portal-based inflammatory infiltrate is almost entirely lymphocytic. Activated and atypical forms may be present, but eosinophils and neutrophils are rare. In acute rejection, the infiltrate is mixed and is composed primarily of lymphocytes, but there are abundant eosinophils, with interspersed neutrophils and plasma cells. The “string of beads” sinusoidal lymphocytosis is also rare in the context of transplant rejection. Bile duct injury can occur in EBV hepatitis, but it is typically less severe than that seen in acute rejection. As mentioned, venular endotheliitis is also characteristic of rejection; however, it can also be seen in EBV hepatitis. Epstein-Barr virus-encoded RNA and/or PCR analysis can be helpful in this situation, but it is also important to note that rejection and EBV infection can coexist in the same posttransplant patient.

**Lymphoproliferative Disorders**

The sinusoidal infiltration of atypical T cells in EBV hepatitis can mimic lymphoproliferative disorders, making it difficult to distinguish the 2 entities on histologic grounds alone. This is particularly true of hepatosplenic T-cell lymphoma (HSTL), which preferentially involves the sinuses of the liver; however, this pattern is also commonly reported in adult human T-cell leukemia, natural killer cell leukemia, and hairy cell lymphoma. Hepatosplenic T-cell lymphoma is a rare, aggressive neoplasm of monomorphic, medium-sized, cytotoxic T cells that infiltrate the liver, spleen, and bone marrow. Microscopically, liver involvement is characterized by diffuse sinusoidal infiltration by intermediate-sized monomorphic lymphocytes with irregular nuclear contours, condensed chromatin, inconspicuous nucleoli, and a moderate amount of pale cytoplasm. Associated sinusoidal dilation is variably prominent (Figure 4, A through C). In contrast to EBV hepatitis, involvement of the portal tracts is typically mild or absent. With disease progression, the lymphocytes may become larger and more pleomorphic. Hepatosplenic T-cell lymphoma has overlapping clinical features with EBV hepatitis with hepatosplenomegaly and a peak incidence in adolescents and young adults, although HSTL shows a male

*Figure 4. Hepatosplenic T-cell lymphoma. A, Sinusoidal infiltration by lymphocytes with minimal portal involvement. B, Atypical lymphoid infiltrate filling and distending the sinuses. C, Neoplastic T cells are predominantly medium sized with irregular nuclei, inconspicuous to small nucleoli, and a moderate amount of pale cytoplasm (hematoxylin-eosin, original magnifications ×20 [A and B] and 40 [C]).*
predominance. In contrast to EBV, patients with HSTL typically have cytopenias without lymphadenopathy, and bone marrow involvement is usually present. Immunophenotypically, the atypical T cells are usually CD3+, CD2+, CD5+, CD7+, CD8+, CD56+, CD57+, and TIA1+. Most HSTL cases are CD4+/CD8-, but CD4+/CD8+ cases are also observed, albeit rarely.18 The HSTL cells are almost always negative for EBER; however, there have been rare reports of EBV positivity, particularly in patients with pleomorphic cytology.20 Ancillary studies can also be used to support a diagnosis of HSTL. T-cell receptor gene rearrangements, identified by PCR, are somewhat limited in specificity and sensitivity but may still be helpful in recognizing T-cell clonality.8,22 Cytogenetics can capitalize on the abnormality in isochromosome arm 7q, which occurs in most cases of HSTL.19

Lymphoproliferative disorders can also be associated with EBV infection in the context of immunosuppressive therapy after transplant. In hepatic posttransplant lymphoproliferative disorder, there is a characteristic, maplike expansion of the portal tracts. Ancillary studies that can assist with diagnosis of posttransplant lymphoproliferative disorder include immunohistochemical staining or in situ hybridization to detect κ and λ light chains, molecular analyses to identify immunoglobulin gene rearrangements, and flow cytometry. Diagnosis of an EBV-related disorder is confirmed by EBER; however, this should be interpreted with caution, given the increased proportion of allograft recipients with rare EBV+ cells.23,24

Drug-Induced Liver Injury

Drug-induced liver injury should always be considered in the differential diagnosis of EBV hepatitis. Diagnosis of drug-induced liver injury is challenging, and it is often difficult to completely exclude other possible causes of liver injury because the histologic features of drug-induced liver injury can emulate any pattern of primary liver disease, including hepatocellular injury, cholestatic injury, mixed hepatocellular-cholestatic injury, steatosis, and vascular lesions. Although the timing of drug use is important, liver toxicity may not become evident for weeks or months after ingestion; drug interactions or dose increases can also influence toxicity.35 Phenytoin hepatotoxicity is most likely to cause a sinusoidal lymphocytosis such as that associated with EBV hepatitis;26,27 however, similar histopathology has also been reported with paraaminosalicylate and with dapsone.28 Laboratory evidence of EBV infection and EBER may be useful in this situation, along with a careful review of the patient’s medications.

CONCLUSIONS

Hepatic involvement with EBV infection is very common, although it is often subclinical. Most cases of EBV hepatitis are self-limited, but severe and potentially fatal disease has been reported. The differential diagnosis is fairly broad and includes autoimmune hepatitis, transplant rejection, lymphomas, and drug-induced liver injury. Although histopathologic features of these disorders sometimes overlap, the characteristic microscopic findings of EBV hepatitis, particularly the diffuse lymphocytic sinusoidal infiltrate in a “string of beads” pattern, is very helpful. The clinical scenario is also an important clue to the diagnosis. Serologic confirmation of EBV infection is also important, and primary infection is indicated by the presence of anti-VCA IgM and absence of anti-EBNA-1 IgG. When positive, EBER in situ hybridization also supports a diagnosis of EBV hepatitis. Unusual clinical and laboratory features such as lack of signs and symptoms of mononucleosis, positive anti-nuclear antibodies, or negative serologies in young children or immunocompromised patients can confound the clinical picture.

References