Epstein-Barr virus: Silent companion or causative agent of chronic liver disease?

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Abstract

The Epstein-Barr virus (EBV) has an important and multifaceted role in liver pathology. As a member of the herpes virus family, EBV establishes a persistent infection in more than 90% of adults. Besides acute hepatitis during primary infection, many clinical syndromes of interest for the hepatologist are associated with EBV infection. The role of EBV in the evolution of chronic hepatitis from hepatotropic viruses is considered. Chronic EBV-associated hepatitis is suspected in immunocompetent adults with compatible serology, suggestive histology and detection of the viral genome in the liver and/or increase of specific circulating cytotoxic T-lymphocytes. EBV is the main cause of post-transplant lymphoproliferative disorders which occur in up to 30% of cases. EBV-driven lymphoproliferative diseases are also recognized in non-immunocompromised patients and liver is involved in up to a third of the cases. Directly implicated in the pathogenesis of different tumors, EBV has a disputable role in hepatocellular carcinoma carcinogenesis. Further research is required in order to establish or reject the role of EBV in human liver cancer. This paper attempts to discuss the range of EBV-associated chronic liver diseases in immunocompetent patients, from mild, self-limiting mononuclear hepatitis to liver cancer.
teins expressed during the lytic infection. Only ten of these proteins are expressed in latently infected B-cells in vitro\[20]. Limited gene expression during latency ensures successful escape from CTL recognition. However, this has led to sophistication of the diagnostic tools for detection of EBV gene products in different tissues. Different types of latency regarding expression of EBV latent genes are established in different diseases and states. EBV-encoded nuclear antigen 1 (EBNA-1) and small RNAs (EBER) are found in Burkitt’s lymphoma, whereas latency type of Hodgkin’s disease is represented by additional expression of the latent membrane proteins (LMP-1 and LMP-2)\[8]. These differences further hamper the diagnosis of EBV-associated disease and challenge both the clinicians and molecular biologists to reveal the causal links.

The classical form of primary EBV infection in the immunocompetent individual is infectious mononucleosis (IM, or first-kiss-disease). Albeit not a challenge for general physicians, in some cases it could be of interest for hepatologists. Almost half of patients with IM have hepatitis, while jaundice is present in 5%-10% of cases\[10,11]. Besides transient liver enzyme elevations, cholestatic pattern of severe hepatitis\[12-14] and even fatal liver failure might complicate the IM\[15-17].

**CHRONIC ACTIVE EBV INFECTION**

Chronic active EBV infection (CAEBV) is thought to be a rare disorder, defined by chronic severe illness, which begins as a primary EBV infection associated with abnormal EBV serology, histological evidence for organ disease (including hepatitis) and demonstration of EBV gene products in tissue. The CAEBV infection might progress to a chronic or recurrent infectious mononucleosis-like disease\[18]. This is a result of a disturbance in the host-virus balance and Th1/Th2 disbalance. It is reported that CAEBV is associated with an aggressive clinical course\[19] but the pathogenesis and pathophysiology of CAEBV are still not well characterized. Patients with iatrogenic, congenital, or acquired immunodeficiency are at increased risk for EBV-associated lymphomas and CAEBV. It seems that CAEBV in Western countries is milder than in Asian countries\[20]. The mild forms from the Western world are characterized by intact immune control of B-cells, relatively low viremia and EBV-specific CTL expansion comparable to seropositive donors. Under the heading of the popular clinical term CAEBV, novel EBV lymphoproliferative diseases (LPDs) have been identified recently in non-immunocompromised hosts, both in Asian and Western countries. Immune senescence in the elderly is also associated with both reactive and neoplastic EBV-driven LPDs\[21]. Almost a third of the patients with some EBV-driven LPDs have liver involvement, and liver failure is an important cause of death in this group\[20].

**EBV-ASSOCIATED CHRONIC HEPATITIS**

Some reports have suggested EBV to be a trigger agent for an autoimmune hepatitis\[22]. It should be kept in mind that liver involvement in CAEBV might mislead even experienced physicians to misdiagnose an autoimmune hepatitis (Aih)\[23]. Nobili et al\[24] identified EBV and hepatitis A virus infections as possible initiating agents in a cohort of Aih type I patients. In similar cases a suppressor T-cell dysfunction and consequent loss of control and raising of ASGRP antibodies have been seen to be involved in the evolution of the viral disease\[25-27].

EBV has been suspected as a probable cause of particular granulomas in the liver\[28] and even in a rare vanishing bile duct syndrome\[29]. In some patients with chronic liver disease caused by a major hepatotropic virus, an infection with other viral agents may be discovered. We previously evaluated patients with chronic hepatitis B and C regarding their EBV serology. Our patients with reactivated EBV infection had lower levels of HBV DNA and higher mean values of serum hepatitis C virus (HCV) RNA respectively, compared to EBV-seropositive patients without reactivation\[30]. Several hypotheses were put forward to explain those results. Reactivation of the EBV-specific T cells leads to significant production of several cytokines, especially interferon-γ (IFN-γ), interleukin (IL)-1, IL-2 and IL-10\[31]. Additionally, the EBV BCRF1 shares high sequence homology with human IL-10 and exerts the same activity. Exogenous IL-10 enhances HCV replication\[32]. IFN-γ inhibits HBV replication in the absence of cell necrosis\[33]. It is unclear why the effect on viral replication was opposite in HBV and HCV cases. Most probably, T cell cross-activation could explain the hepatitis virus reactivation.

The latter conclusion has been drawn from a small group of patients whose EBV reactivations preceded HBV flares\[34]. Sugawara et al\[34] have shown in vitro that EBNA1 promotes HCV replication. Speculative viral or immune interference in cases of chronic hepatitis B or C and EBV infection needs further research.

Bertolini et al\[35] investigated an experimental model (nu/nu mice) of chronic EBV hepatitis with typical transsinusoidal lesions after inoculation of EBV-infected B-cells. The study provided evidence for EBV replication in a very rare proportion of hepatocytes. Despite lack of evidence, we might speculate that infection of non-lymphoid cells is possible via a specific integrin-dependant mechanism\[36]. Hesitant reports have linked EBV to chronic hepatitis in non-immunocompromised humans. The existence of EBV-associated liver disease is still accepted with caution as EBV has not been detected in human hepatocytes\[37]. Specific latent antigens as well as EBER transcripts are detected in infiltrating lymphocytes. It has been proposed that liver cells suffer from a form of collateral damage\[38]. Chronic hepatitis might also be induced by a soluble Fas-ligand, tumor necrosis factor and IFN-γ\[39,40]. In most cases the infiltrating lymphocytes are CD8+ CTLs. It has also been found that activated CD8+ cells might be selectively trapped in the liver as they are bound to specific adhesive molecules expressed by Kupffer cells\[41]. In the last year, several studies have tried to lift the curtain regarding chronic hepatitis associated with EBV infection. Drebber et al\[42] proposed a list

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of criteria to establish the diagnosis: presence of suggestive histopathological features, serological profile and detection of viral genome in the liver tissue. In spite of some methodological limitations, this study addressed the necessity of new scoring systems regarding the possible EBV-associated chronic liver disease. Their work revived the interest in this issue both for pathologists and molecular hepatologists [43]. The critics of a hypothesis for chronic EBV liver disease in non-immunocompromised patients discuss so called “incidental virus” or amplification of the genome in circulating B cells which turn up in the liver. The existence of acute mononuclear hepatitis during primary EBV infection has been already accepted. Hence, it is most likely that the reactivation leading to liver damage occurs whether the infected lymphocytes are incidentally or intentionally in the liver. We previously discussed cases of EBV-associated chronic hepatitis in patients with reactivated EBV infection and increased specific CTL-response to a lytic EBV-epitope compared to healthy controls [44]. We presumed frequent reactivations in these patients because we had found an increased percentage of terminally differentiated CD28(-) CD27(-) CD8(+) T cells, suggestive of chronic antigen stimulation and replicative senescence. Diminished expression of co-stimulatory molecules CD28 and CD27 could further compromise CD8+ reactivation and characterize these terminally differentiated cells as more resistant to apoptosis [45,46]. Focused T-cell pull with low expression of CD28 and CD27 has low ability to control reactivations and is a typical finding in an elderly group. Surprisingly, very similar changes were found in younger patients under chronic cytomegalovirus and EBV antigen stimulation [47].

We surmise that a chronic liver disease might be a manifestation of a chronic EBV infection with frequent reactivations and persistent, moderate or low level of viral load. Based on recent studies and cases we can propose the expansion of Drebber’s criteria by adding recurrent EBV reactivations, increased circulating EBV-specific CTLs, and increased CD38 B-cell expression. We are also inclined to accept as additional clinical signs the increase of LDH levels, mild spleen enlargement and tendency for thrombocytopenia.

### LIVER TRANSPLANTATION AND EBV INFECTION

EBV infection is the main cause of post-transplant lymphoproliferative disease (PTLD). The incidence of PTLD ranges from 0.5% to 30% depending on the organ being transplanted, the EBV status of the transplant recipient and donor, and the therapies used to achieve immunosuppression [48,49]. EBV seronegativity at the time of transplant and pediatric age are predisposing factors. The disorder occurs commonly in combined liver-kidney transplants, followed by cardiac, liver, lung, and then kidney transplants. In addition, constitutional factors such as cytokine gene polymorphism may play a predisposing role. Little is known about the chronic carrier state and its relation to late PTLD. Most pediatric liver transplant candidates are EBV-naïve when listed for orthotopic liver transplantation (OLT). Those immunosuppressed, EBV-naïve children, who receive an adult EBV-positive liver, are unable to adequately control EBV primary infection, hamper viral replication and avoid lymphoproliferation [50]. For this reason, PTLD has become one of the major threats in transplantation, complicating the course of up to 10% of pediatric liver graft recipients, with a mortality reported up to 50% [51]. All data support the recommendation of strict EBV monitoring in the first months after the transplantation. However, little is known about late and chronically persistent infection. Helpful insights into how often and how long we should monitor viremia during follow-up are insufficient. D’Antiga et al. [52] revealed that none of their pre-OLT seropositive patients had sustained positivity of EBV serology or polymerase chain reaction (PCR) for 6 mo or longer, versus as many as 65% of those who were EBV-negative pre-OLT. A prolonged EBV viral load was strictly related to pre-OLT immunity against the virus and appeared to be associated with the occurrence of PTLD [52]. In fact, it is well established that persistence of EBV is a risk factor for development of lymphomas [53]. Patients who demonstrate a sustained viral detection are at risk for the development of late PTLD which implies necessity for preliminary testing as well as for long-term EBV infection follow-up. The development of molecular genetic diagnosis for EBV (PCR) means that progressive disease, or PTLD, may be prevented by preemptive reduction of immunosuppression in response to rising EBV titers.

### EBV AND LIVER CANCER

EBV was the first human virus directly implicated in carcinogenesis. Since its discovery it has been considered as a major player in the development of a wide range of cancers both in immunocompetent and immunocompromised individuals. Recent studies have concluded EBV itself or infected cell clones might promote replication of the HCV [54,55]. Presumably, EBNA1 is responsible for supporting HCV replication, suggesting that EBV may be involved in the development of hepatocellular carcinoma (HCC) [54]. The detection of EBV gene products in HCC additionally supports this assumption. Sugawara et al. [55] reported a higher amount of EBV DNA in HCV-positive HCC compared to HBV-associated HCC. EBV-infected cells support HCV replication better than uninfected cells, suggesting that EBV may act as a helper virus to promote HCV replication in the HCV-positive HCCs, especially in Japanese patients. Li et al. [56] found that almost 30% of liver cancers harbored EBV DNA (≥8% in the control group, P < 0.05) suggesting that the herpes virus might be involved in hepatocellular carcinogenesis in China. However, studies from Western countries do not confirm the hypothesis that EBV may promote the development of HCC. Akhter et al. [57] examined cancer tissues of 31 non-cirrhotic, HCC patients. None of the tumor samples were found to be positive for EBV DNA. Being unable to con-
firm an association between EBV and infection with hepatitis viruses in HCC, the authors suggested environmental or genetic factors which could predispose Chinese and Japanese patients to develop EBV-infected HCC. On the other hand, a possible source of detected EBV DNA might be the infiltrating lymphocytes. Furthermore, a study from The Netherlands concluded that the absence of epithelial-specific B-ARF1 transcripts and other EBV transcripts and proteins in EBV DNA PCR-positive cases argues strongly against a role for EBV in HCC. However, weak signals for EBV DNA in 5 of 16 HCCs (31.25%) and in two of four HCV-related hepatitis cases were detected and actually no EBV DNA was found in non-neoplastic and normal liver tissue, using DNA PCR-Enzyme Immunoassay for the detection of the BamHI W repeat fragment of EBV. The weak positivity of EBV DNA in some liver tissues was explained by amplification of EBV DNA in the lymphoid infiltrate or blood, reflecting a high EBV DNA load in these patients. Chen et al showed that circulating EBV-positive peripheral B cells express B-ARF0 transcripts which could also explain the negative BamHI A rightward transcripts RNA in situ hybridization results. Moreover, we cannot exclude the presence of EBV-positive lymphocytes or EBER-positive hepatocytes in the larger amounts of tissue, as most studies usually test routine histology sections (5μm thick paraffin-embedded or cryosections). This fact raises the question whether usual techniques such as immunohistochemistry and in situ hybridization lack enough power to detect the virus in the liver regardless of its place of persistence - infiltrating lymphocytes, hepatocytes or Kupffer and other liver cells. We would welcome better evidence for or against the causative (and/or supportive) role of EBV in human HCC carcinogenesis.

Underservedly neglected within hepatological society, EBV-associated chronic liver disease demonstrates the challenges in discovering the intimate mechanism of viral-host interactions leading to different clinical syndromes. Since proven to induce acute hepatitis during IM, to affect the liver in a PTLD setting, possibly to contribute to liver carcinogenesis, it would not be surprising if EBV causes chronic hepatitis. Undoubtedly, follow-up of patients is required to reveal the long-term complications of EBV-associated liver disease before we decide that this viral infection belongs in a group of meaningless companions of cryptogenic disease.

REFERENCES

12 Henedi TB, Koff RS. Cholestatic hepatitis induced by Epstein-Barr virus infection in an adult.Dig Dis Sci 2003; 48: 539-541
15 Papatheodoridis GV, Dalladetsima JK, Kavalierou L, Kapranos N, Tassopoulos NC. Fulminant hepatitis due to Epstein-Barr virus infection. J Hepatol 1995; 23: 348-350
25 Nobili V, Comparcola D, Sartorelli MR, Devito R, Marcellini M. Autoimmune hepatitis type 1 after Epstein-Barr virus in-


28 Aceti A, Mura MS, Babudieri S, Bacciu SA. A young woman with hepatitis after a sore throat. Lancet 1995; 346: 1603


32 Jabs WJ, Wagner HJ, Schlenke P, Kirchner H. The primary and memory immune response to Epstein-Barr virus infection in vitro is characterized by a divergent production of IL-1beta/IL-6 and IL-10. Scand J Immunol 2000; 52: 304-308


34 Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999; 284: 825-829


42 Mehall WZ, Juedes AE, Crisey IN. Selective retention of activated CD8(+) T cells by the normal liver. J Immunol 1999; 163: 3202-3210


46 Dunne PJ, Faint JM, Gudgson NH, Fletcher JM, Plummet FJ, Soares MH, Hidalgo AD, Annels NE, Richardson AB, Salmon M, Akbar AN. Epstein-Barr virus-specific CD8(+) T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. Blood 2002; 100: 953-940


50 McDiarmid SV. Management of the pediatric liver transplant patient. Liver Transpl 2001; 7: 577-586


56 Li W, Wu BA, Zeng YM, Chen GC, Li XX, Chen JT, Guo YW, Li MH, Zeng Y. Epstein-Barr virus in hepatocellular carcinoma tissues from hepatitis C-positive patients. J Gastroenterol 2004; 39: 538-547

57 zur Hausen A, van Beek J, Bloemena E, ten Kate FJ, Meijer CJ, van den Brule AJ. No role for Epstein-Barr virus in Dutch hepatocellular carcinoma: a study at the DNA, RNA and protein levels. J Gen Virol 2003; 84: 1863-1869


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