ANNALS of Hepatology

CASE REPORT

January-February, Vol. 12 No.1, 2013: 147-151

Autoimmune hepatitis type-2 and Epstein-Barr virus infection in a toddler: art of facts or an artifact?

Aglaia Zellos,* Vana Spoulou,* Eleftheria Roma-Giannikou,* Ourania Karentzou,** George N. Dalekos,*** Maria Theodoridou*

* First Department of Pediatrics, Aghia Sophia Children's Hospital, University of Athens School of Medicine, Athens, Greece.
** Department of Pathology, Aghia Sophia Children's Hospital, University of Athens School of Medicine, Athens, Greece.
*** Department of Medicine, Thessaly University, School of Medicine, Larissa, Greece.

ABSTRACT

Epstein-Barr virus (EBV) can cause frequently asymptomatic (or anicteric) and self-limited hepatitis, while occasionally may result in considerable cholestatic hepatitis. Herein, we describe the case of a previously healthy toddler (26 month old girl) with prolonged cholestasis, elevated serum transaminases, EBV serology compatible with recent EBV infection and positive anti liver kidney microsomal antibody type 1 which is characteristic of new-onset autoimmune hepatitis type 2. Liver biopsy was also typical of autoimmune hepatitis as attested by the presence of portal inflammation with predominant T-lymphocytes and plasma cells and interface hepatitis. Persistent EBV-related hepatitis was excluded by the absence of viral inclusions and steatosis on liver specimens and negative liver EBV-PCR. In conclusion, our case strongly suggests that in children with prolonged cholestatic hepatitis, positive EBV serology cannot exclude the presence of other causes of liver disease. In this context, autoimmune hepatitis should be considered as an alternate diagnosis, particularly when there is specific liver-related autoantibody detection. In such conditions, liver biopsy seems mandatory in an attempt to achieve a correct and timely diagnosis of a potentially catastrophic disease as autoimmune hepatitis. Although some cases of autoimmune hepatitis type 1 following EBV infection have been reported in adults, to the best of our knowledge, the present case of autoimmune hepatitis type 2 after EBV infection represents the first case in children ever reported in the English literature.

Key words. Autoimmune liver diseases. Children. Cholestasis. Epstein-Barr infection. Liver kidney microsome antibodies.

INTRODUCTION

Epstein-Barr virus (EBV) causes a wide spectrum of diseases ranging from mild to severe infectious mononucleosis to B-cell lymphoma. Most frequently, EBV induces self-limiting hepatitis with mild to moderate elevations of aminotransferases whereas rarely considerable cholestasis accompanied by increased bilirubin levels can be observed.[1]

The initiation and persistence of autoimmune diseases have not been elucidated, although persistent inflammation, infection, apoptosis, environmental exposure and genetic susceptibility have been implicated. Accordingly, acute or latent EBV infection has already been suggested in autoimmunity process in adults including autoimmune hepatitis (AIH),[2,3] an unresolving, progressive liver disease characterized by hypergammaglobulinaemia, circulating autoantibodies, association with human leukocyte antigens (HLA), interface hepatitis on liver histology and a favourable response to immunosuppression.[4-6] However, the involvement of EBV in autoimmunity induction in children has been rarely reported.[7,8]

After a written informed consent from parents, we describe a previously healthy toddler with new-onset AIH-type-2 (AIH-2) and positive EBV serology who presented with cholestatic hepatitis. Our report illustrates the importance of liver biopsy in achieving a correct and timely diagnosis by distinguishing EBV infection from AIH-2, as treatment options for each of the above clinical entities differ significantly.

Correspondence and reprint requests: George N. Dalekos, M.D., Ph.D.
Professor of Medicine, Head, Department of Medicine and Research Laboratory of Internal Medicine, University of Thessaly, School of Medicine
Biopolis, Larissa, 41110 Larissa, Greece
Tel: +30-241350-2285. Fax: +30-241350-1863
E-mail: dalekos@med.uth.gr

Manuscript received: February 21, 2012.
Manuscript accepted: May 23, 2012.

CASE REPORT

A previously healthy 26 month old girl was admitted to our department because of jaundice, abdominal pain, clay-colored stools for 4-days and a preceded 10-days course of fever up to 39 °C. There was no family history of consanguinity, autoimmune diseases, chronic liver diseases or cystic fibrosis. Complete vaccinations against hepatitis A (HAV) and B viruses (HBV) had been performed. On physical examination, she was afebrile with normal development but marked scleral icterus and pruritus were observed. Head and neck examination was normal without palpable lymphadenopathy. The abdomen was distended with tender hepatomegaly without splenomegaly. Respiratory, cardiac, skin and neurological investigations were unrevealing.

On admission:

- Total bilirubin: 7.7 mg/dL (direct: 4.3 mg/dL).
- AST 1962 IU/L (upper normal limit, UNL: 60IU/L).
- ALT 2,334 IU/L (UNL: 45IU/L).
- γ-GT 303IU/L (UNL: 50IU/L).
- Alkaline phosphatase 1,004 IU/L (UNL: 2,50 IU/L).
- Total protein 7.8 g/L.
- Albumin 5.2 g/L (range: 3.4-4.8).
- ESR 29 mm/h.
- Prothrombin time 15.6 sec (control 11sec).
- Factor VII 32%.
- Serum IgG was 1,140 mg/dL (normal 708-1622).
- IgA 72 mg/dL, IgM 94 mg/dL.

Serum amylase and lipase levels were normal. Stool bacterial cultures tested negative for pathogens. Direct and indirect Coombs tests as well as testing serology for celiac disease-related autoantibodies, HAV, HBV and hepatitis C virus (HCV), enteroviruses, parvovirus, and human herpes viruses (HHV) 1, 2 and 6 were negative. Blood testing by PCR for HCV, cytomegalovirus (CMV), leptospira species and adenovirus was also negative. Testing for EBV-VCA IgM and IgG isotype specific antibodies was strongly positive (IgM > 200 IU; IgG 47I U; both cut-off >10 IU) though serum EBV-PCR was negative (cut-off: 600 copies/mL). Liver autoimmune serology by indirect immunofluorescence revealed negativity for smooth muscle antibodies (SMA, positive titer ≥ 1:20), antinuclear antibodies (ANA, positive titer ≥ 1:20) and antimitochondrial antibodies (AMA, positive titer ≥ 1:20) but positivity for antibodies against liver kidney microsomal type-1 by indirect immunofluorescence (anti-LKM1; titer 1:160; positive titer ≥ 1:10), which was further confirmed by molecularly based assays like ELISA and Western blot as described previously.[4,9-11] Serum copper, ceruloplasmin and 24 h urinary copper collection were within normal limits. α_1-antitrypsin phenotype was MM and thyroid function was normal. HLA type analysis revealed HLA-A2, 24, HLA-B13, 49 and HLA-DR13 and DR7. Hepatobiliary ultrasound showed normal liver parenchyma, increased liver size, and normal intra-and extra hepatic biliary tree. A percutaneous liver biopsy was performed after infusion of fresh frozen plasma and recombinant Factor VII. The liver lobular architecture was preserved but lobular hepatitis, portal inflammation with predominant T-lymphocytes and plasma cells and interface hepatitis were obvious (Figure 1). There was considerable fibrosis between portal tracts and the Histology Activity Index Score (12) was 14 (inflammation 12 and fibrosis 2). No biliary or other lesions were observed. Liver tissue testing by PCR was negative for EBV.

AIH-2 diagnosis was made based on the descriptive criteria for the diagnosis of AIH published since 1999 by the International AIH Group[13] in a female child with highly increased transaminase levels, anti-LKM1 positivity, absence of HBV and HCV viral markers, absence of alcohol use and other toxic agents, typical liver histology and absence of EBV genome on liver tissue. At the time of liver biopsy, EBV-VCA IgM and IgG remained positive (195IU and 185IU, respectively). Repeated EBV serology 10 days later, provided strong evidence for an EBV recent-past infection as attested by negativity for EBV-VCA IgM, Early Antigen (EA) IgG and

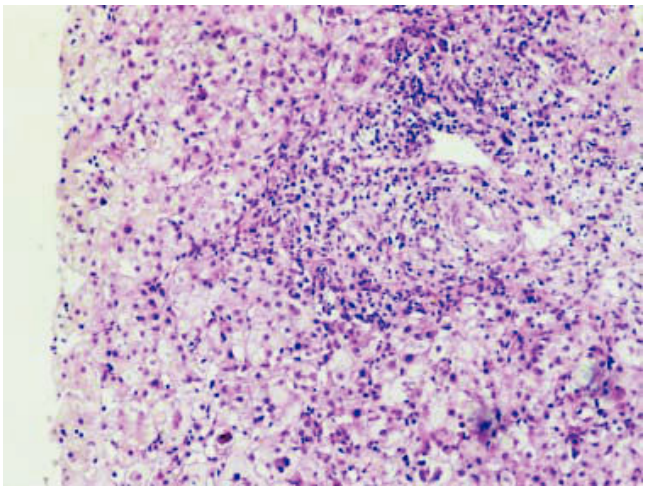

***Figure 1**. Percutaneous liver biopsy specimen showing portal inflammation with increased plasma cells and interface hepatitis.*

EBV-PCR while, EBV-VCA IgG and EBNA tested positive [> 200 IU and 5.8 IU (> 3 IU positive), respectively].

Immediate treatment with prednisolone 2 mg/kg/day plus azathioprine 2 mg/kg/day and fat soluble vitamins resulted in improvement of jaundice and normalization of serum transaminases after 8-weeks of therapy. Prednisolone was tapered over 3-months. Serum anti-LKM1 titer decreased to 1:80, 6-months later. Two years after treatment, the patient is still on azathioprine maintenance monotherapy (2 mg/kg/day) with complete clinical and biochemical remission, negative serum EBV-PCR and positive anti-LKM1 (titer 1:40). The family has refused a follow-up liver biopsy.

DISCUSSION

We report a case of acute onset of AIH-2 following recent-past EBV infection in a healthy toddler presenting with icteric cholestatic hepatitis. The very high transaminase levels and the presence of cholestasis led to a full diagnostic evaluation upon hospital admission. The concomitantly positive EBV-IgM, EBV-IgG, and anti-LKM1 perplexed the differential diagnosis. Though the possibility of false-positive EBV serology due to a non-specific B-cell activation cannot be excluded safely, the rising values of EBV-IgG and the declining values of EBV-IgM antibodies, along with positivity for EBV-EA and negativity for serum EBV-PCR are indicative of recent-past EBV infection which has provoked the development of AIH-2 in a genetically susceptible female with DR7 haplotype. According to a previous report by Ma, *et al.*[14] children carrying the HLA-DR7 (DRB1*0701) haplotype are more susceptible to AIH-2 and develop more aggressive disease with more severe prognosis. Interestingly, our patient also possesses HLA-DR13 which in South American children (DRB1*1301 allele), predisposes to AIH-1 and to persistent infection with the endemic HAV.[15,16]

Liver biopsy is a valuable tool in diagnosis, prognosis and therapeutic management decisions in patients with liver disease and of outmost importance in the case of AIH.[5,6,9,17,18] Indeed, the role of liver biopsy has proved crucial in our case since liver histology resulted in the distinction between two entirely different clinical entities, the EBV-related hepatitis and AIH-2, in which opposite treatment options are required. Persistent EBV viral hepatitis was excluded by the absence of viral inclusions on histology and negative liver EBV-PCR. A twice negative serum EBV-PCR was in concordance with these findings. AIH-2 diagnosis was based on the original and simplified criteria published by the International AIH Group as attested by the elevated serum aminotransferases, positive anti-LKM1 titers, typical liver histology and absence of HBV and HCV.[5,13] Clinical and biochemical response to immunosuppression as opposed to serious flares which have been recorded in viral infections due to HBV, HCV and delta virus or other hepatotropic viruses,[9,13] further supports our diagnosis. Interestingly, hypergammaglobulinemia although included in both original and simplified diagnostic criteria of AIH[5,13] was not found in our patient. Similarly, a previous study on 52 children with AIH showed that 20% of them (half anti-LKM1 positive) had normal serum IgG, indicating that such a finding does not exclude AIH diagnosis in childhood.[19] Indeed, a limitation of all these scoring systems is that they have been produced for adult patients and need to be adapted to children.[9]

Prompt initiation of immunosuppression initially with prednisolone followed by azathioprine 2-weeks later, resulted in complete normalization of transaminses 2-months later. However, since children with AIH-2 tend to relapse frequently when therapy is discontinued[9,19] long term prognosis of our patient is uncertain and may require lifelong treatment with azathioprine and even small doses of prednisolone.

It seems rational that in our case, EBV infection unmasked or triggered AIH-2 development in a susceptible child. Single case reports have recorded AIH-1 onset after infection with HAV, EBV, HHV-6 and measles in adults.[3,16,20-22] Specifically for EBV, Vento, *et al.*[21] have reported the onset of AIH-1 in two out of 7 susceptible adults after EBV infection while Cabibi,[22] reported another similar AIH-1 adult case. Additionally, AIH-2 has been reported in adults with HCV after treatment with interferon-a[23] but also in an adult after acute HCV infection even after viral clearance.[24] However, to the best of our knowledge previous reports published in the English literature regarding the AIH-2 onset after EBV infection in children are missing. The only report in children concerning the onset of AIH-1 (not AIH-2) after EBV infection is available in the Japanese language.[25] From the pathogenetic point of view, the interplay between viruses and susceptible hosts may result in the clinical development of autoimmunity either by cytokines release which activate autoreactive T-cells and modify antigen processing and activation or by molecular mimicry.[3,4,20,26] In this

context, Pender[27] recently proposed that latent EBV infected autoreactive memory B-cells, lodge to the target organ and act as antigen-presenting cells attracting CD4+ T-lymphocytes that fail to undergo apoptosis, as they receive a co-stimulatory survival signal from infected B-lymphocytes.

In conclusion, this report indicates that in children with prolonged cholestatic hepatitis, positive EBV serology cannot exclude the presence of severe liver diseases and therefore, AIH should be considered as an alternate diagnosis, particularly when there is specific liver-related autoantibody detection like anti-LKM1. In such conditions, liver biopsy seems mandatory in an attempt to achieve a correct and timely diagnosis of a potentially catastrophic liver disease as AIH-2. Although some cases of AIH-1 following EBV infection have already been published in adults, the present report of AIH-2 after EBV infection represents the first case in children ever reported in the English literature.

ABBREVIATIONS

- **AST:** aspartate aminotransferase.
- **ALT:** alanine aminotransferase.
- **ESR:** erythrocyte sedimentation rate.
- **ANA:** antinuclear antibody.
- **ASMA:** anti-smooth muscle antibody.
- **Anti-LKM:** anti-liver kidney microsomal antibody.
- **AMA:** anti-mitochondrial antibody.
- **pANCA:** perinuclear anti-neutrophil cytoplasm antibodies.
- **EBV-VCA:** Epstein-Barr viral capsid antigen.
- **EBNA:** Epstein-Barr Nuclear Antigen.

SUPPORTIVE FOUNDATIONS

Nothing to declare.

REFERENCES

1. Shaukat A, Tsai HT, Rutherford R, Anania FA. Epstein-Barr virus induced hepatitis: An important cause of cholestasis. *Hepatol Res* 2005; 33: 24-6.
2. Niller HH, Wolf H, Minarovits J. Regulation and dysregulation of Epstein-Barr virus latency: implications for the development of autoimmune diseases. *Autoimmunity* 2008; 41: 298-328.
3. Vento S, Cainelli F. Is there a role for viruses in triggering autoimmune hepatitis? *Autoimmunity Rev* 2004; 3: 61-9.
4. Dalekos GN, Zachou K, Liaskos C, Gatselis N. Autoantibodies and defined target autoantigens in autoimmune hepatitis: an overview. *Eur J Intern Med* 2002; 13: 293-303.
5. Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, Bittencourt PL, et al. International Autoimmune Hepatitis Group. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; 48: 169-76.
6. Zachou K, Gatselis N, Papadamou G, Rigopoulou EI, Dalekos GN. Mycophenolate for the treatment of autoimmune hepatitis: prospective assessment of its efficacy and safety for induction and maintenance of remission in a large cohort of treatment-naïve patients. *J Hepatol* 2011; 55: 636-46.
7. McClain MT, Poole BD, Bruner BF, Kaufman KM, Harley JB, James JA. An altered immune response to Epstein-Barr nuclear antigen 1 in pediatric systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 360-8.
8. Sevilla J, del Carmen Escudero M, Jiménez R, González-Vicent M, Manzanares J, García-Novo D, Madero L. Severe systemic autoimmune disease associated with Epstein-Barr virus infection. *J Pediatr Hematol Oncol* 2004; 26: 831-3.
9. Mieli-Vergani G, Heller S, Jara P, Vergani D, Chang MH, Fujisawa T, González-Peralta RP, et al. Autoimmune hepatitis. *J Pediatr Gastroenterol Nutr* 2009; 49: 158-64.
10. Gatselis NK, Georgiadou SP, Tassopoulos N, Zachou K, Liaskos C, Hatzakis A, Dalekos GN. Impact of parietal cell autoantibodies and non-organ-specific autoantibodies on the treatment outcome of patients with hepatitis C virus infection: a pilot study. *World J Gastroenterol* 2005; 11: 482-7.
11. Renaudineau Y, Dalekos GN, Gueguen P, Zachou K, Youinou P. Anti-α-actinin antibodies cross-react with anti-ssDNA antibodies in active autoimmune hepatitis. *Clin Rev Allergy Immunol* 2008; 34: 321-5.
12. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431-5.
13. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, et al. International Autoimmune Hepatitis Group Report: review criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; 31: 929-38.
14. Ma Y, Bogdanos DP, Hussain MJ, Underhill J, Bansal S, Longhi MS, Cheeseman P, et al. Polyclonal T-cell responses to cytochrome P450IID6 are associated with disease activity in autoimmune hepatitis type 2. *Gastroenterology* 2006; 130: 868-82.
15. Pando M, Larriba J, Fernandez GC, Fainboim H, Ciocca M, Ramonet M, Badia I, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. *Hepatology* 1999; 30: 1374-80.
16. Czaja AJ, Souto EO, Bittencourt PL, Cancado EL, Porta G, Goldberg AC, Donaldson PT. Clinical distinctions and pathogenic implications of type 1 autoimmune hepatitis in Brazil and the United States.
17. Papamichalis PA, Zachou K, Koukoulis GK, Veloni A, Karacosta EG, Kypri L, Mamaloudis I, et al. The revised international autoimmune hepatitis score in chronic liver diseases including autoimmune hepatitis/overlap syndromes and autoimmune hepatitis with concurrent other liver disorders. *J Autoimmune Dis* 2007; 4: 3.
18. Gatselis NK, Zachou K, Papamichalis P, Koukoulis GK, Gabeta S, Dalekos GN, Rigopoulou EI. Comparison of simplified score with the revised original score for the diagnosis of autoimmune hepatitis: a new or a complementary diagnostic score? *Dig Liver Dis* 2010; 42: 807-12.
19. Gregorio GV, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, Mowat AP, et al. Autoimmune hepatitis

in childhood: a 20 year experience. *Hepatology* 1997; 25: 541-7.
20. Bogdanos DP, Dalekos GN. Enzymes as target antigens of liver-specific autoimmunity: the case of cytochromes P450s. *Curr Med Chem* 2008; 15: 2285-92.
21. Vento S, Guella L, Mirandola F, Cainelli, Di Perri G, Solbiati M, Ferraro T, et al. Epstein-Barr virus as a trigger for autoimmune hepatitis in susceptible individuals. *Lancet* 1995; 346: 608-9.
22. Cabibi D. Autoimmune hepatitis following Epstein-Barr virus infection. *BMJ Case Rep* 2008; 2008: bcr0620080071.
23. Dalekos GN, Wedemeyer H, Obermayer-Straub P, Kayser A, Barut A, Frank H, Manns MP. Epitope mapping of cytochrome P4502D6 autoantigen in patients with chronic hepatitis C during alpha-interferon treatment. *J Hepatol* 1999; 30: 366-75.
24. Vento S, Cainelli F, Renzini C, Concia E. Autoimmune hepatitis type 2 induced by HCV and persisting after viral clearance. *Lancet* 1997; 350: 12.
25. Nakajima S, Umebayashi H, Kurosawa R, Imagawa T, Katakura S, Mori M, Aihara Y, et al. A case of autoimmune hepatitis needed to be differentiated from EBV hepatitis, in that the histology of liver biopsy specimen was useful for diagnosis. *Nihon Rinsho Meneki Gakkai Kaishi* 2005; 28: 154-8.
26. Rigopoulou EI, Dalekos GN. Autoimmune hepatitis: of host and pathogen. *Hepatology* 2008; 47: 2147-8.
27. Pender MP. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. *Trends Immunol* 2003; 24: 584-8.