Clinical Case Report

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Chronic hepatitis E virus infection in a cirrhotic patient

A case report

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Abstract

Rationale: Acute hepatitis E virus (HEV) infections are usually self-limiting in immunocompetent patients. HEV persistence has been described only in immunosuppressed patients such as solid-organ transplant recipients, patients with hematological diseases, or patients with human immunodeficiency virus (HIV) infection.

Patient concerns: A 61-year-old patient was admitted in hospital for jaundice and asthenia.

Diagnoses: The patient had underlying cirrhosis and developed a chronic HEV infection.

Intervention: Ribavirin therapy was initiated.

Outcomes: Ribavirin therapy for 12 months allowed the clearance of the virus and HEV viral load remained undetectable thereafter. This patient had taken no immunosuppressive drugs, was not suffering from any autoimmune disease and was not infected with HIV. We studied the patient's anti-HEV immune response months after the viral clearance. His peripheral blood mononuclear cells (PBMC) were stimulated in vitro by HEV peptides. The patient had a mild T lymphopenia, but polyclonal stimulation of PBMC showed a robust T cell response. The response of his anti-HEV specific interferon-γ producing T cells was low.

Lessons: Other studies are now needed to identify the population with a chronic evolution of HEV infection despite no apparent immunodepression.

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, ELISpot = enzyme-linked immunospot, HAV = hepatitis A virus, HBV = hepatitis B virus, HCV = hepatitis C virus, HEV = hepatitis E virus, HIV = human immunodeficiency virus, IC = immunocompetent, IFN-γ = interferon γ, Ig = immunoglobulin, NASH = nonalcoholic steatohepatitis, PBMC = peripheral blood mononuclear cells, RNA = ribonucleic acid, SOT = solid-organ transplant.

Keywords: chronic infection, cirrhosis, hepatitis E virus

1. Introduction

Hepatitis E virus (HEV) has 4 major genotypes that infect humans worldwide. Genotype 3 is frequently found in industrialized countries and is zoonotically transmitted. Acute HEV infections are generally asymptomatic and spontaneously resolve in immunocompetent (IC) patients.[1] Liver failure associated with HEV infections can occur in patients with underlying liver disease.[1] Chronic HEV genotype 3 infections, in which a detectable virus load persists for >3 months,[2] can occur in immunocompromised such as solid-organ transplant (SOT) recipients,[3] those with hematological diseases,[1] or human immunodeficiency virus (HIV) infections whose CD4+ T cell count is below 200 mm^{-3}.[4] Untreated chronic hepatitis can rapidly lead to liver fibrosis and cirrhosis.[1] The immunological factors implicated in virus clearance are still poorly understood but the cellular response appears essential for HEV eradication. Several studies have shown that SOT patients chronically infected with genotype 3 have poorer anti-HEV immune responses than patients with a resolved infection.[5,6] Recent data indicate that ribavirin therapy efficiently cures 78% of chronically infected SOT patients.[7] This report describes the case of a cirrhotic patient suffering from an acute HEV infection that evolved toward chronicity. We examined the patient's specific anti-HEV immune response by quantifying his specific interferon-γ (IFN-γ) producing T cells.

2. Methods

2.1. Patients

We collected peripheral blood mononuclear cells (PBMC) from this cirrhotic patient 5 months after virus clearance by ribavirin therapy. Because he did not belong to a population known to

Editor: Esaki M. Shankar.

The authors declare no conflicts of interest.

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Medicine (2017) 96:39(e7915)

Received: 12 January 2017 / Received in final form: 13 July 2017 / Accepted: 3 August 2017

http://dx.doi.org/10.1097/MD.0000000000007915

suffer from chronic HEV infection, we investigated not only his specific anti-HEV immune responses, but also those of other HEV-infected patients. We also studied PBMC samples collected at the acute phase of infection from 21 patients: 7 IC patients (median age: 64), 5 SOT patients who spontaneously cleared the virus (median age: 43), and 9 SOT patients who evolved toward a chronic infection previously described[6] (median age: 43). The study was approved by the institutional review board at Toulouse University hospital and all patients gave their written consent.

2.2. ELISpot assay

Specific anti-HEV responses were analyzed by enzyme-linked immunospot (ELISpot) and IFN-γ producing cells were quantified after antigen stimulation in vitro as previously described.[6] Briefly, PBMC samples were incubated with a pool of 76 HEV genotype 3 (Genbank accession number EU495148) open reading frame-2/3 peptides (15-mers, 5 amino-acid overlaps, Genscript, Piscataway, NJ) or with anti-CD3 and anti-CD28 antibodies (clones HIT3a and 28.2, respectively, BD Biosciences, 0.5 μg/mL each) at 37°C for 36 hours (duplicates wells, final volume 60 μL). The IFN-γ ELISpot assays were performed following manufacturer protocol (Diaclone kit); average number of IFN-γ producing cells for duplicate wells were calculated. Anti-HEV and anti-CD3/CD28 IFN-γ responses were calculated after subtracting nonspecific responses from background wells. The Mann-Whitney *U* test was used for statistical analysis, calculations were performed using GraphPad Prism software.

3. Case report

A 61-year-old man was hospitalized on July 21, 2012 for jaundice and asthenia. He was suffering from obesity (body mass index = 30), type 2 diabetes, hypercholesteremia, and a stable stented ischemic cardiopathy. He was treated with acetylsalicylic acid, clopidogrel, enalapril, metformin, insulin, pravastatin, rabeprazole, and celiprolol. He did not report any alcohol consumption or travel. Blood analysis showed elevated alanine/aspartate aminotransferase (ALAT: 15 N and ASAT: 10 N) and gamma-glutamyltransferase (10 N) activities, normal alkaline phosphatase activity, a total bilirubin of 55 μmol/L, albuminemia of 27 g/L, and an international normalized ratio of 1.14. Blood cell and platelet counts were normal. His only recent treatment before hospitalization was amoxicillin and clavulanic acid for 1 week for flulike symptoms. We found no markers of recent hepatitis A virus (HAV), hepatitis B virus (HBV), or hepatitis C virus (HCV) infections (ie, anti-HAV IgM, HBsAg, anti-HBs, anti-HBc, or anti-HCV). Serological tests for acute Epstein-Barr virus or cytomegalovirus infections were negative. Autoantibody tests were negative (antinuclear, antitype 2 mitochondria, anti-smooth muscle tissue, and anti-LKM1 antibodies). His cupremia, cupruria, and ceruloplasmin were all near normal. Magnetic resonance imaging and echography showed steatosis and a dysmorphic liver. Esophago-gastro-duodenoscopy revealed fundal gastritis with a mosaic appearance evocating portal hypertensive gastropathy. Initial diagnosis was drug-induced hepatitis that decompensated nonalcoholic steatohepatitis (NASH) cirrhosis. The patient was readmitted on September 24, 2012 with persistent hepatic cytolysis (ALAT: 5 N), asthenia, jaundice, and development of ascites. The ascetic fluid was turbid: cell count, 100 mm^{-3}; total protein, 7 g/L. Further investigation showed that his blood plasma tested positive for anti-HEV immunoglobulin M (IgM), anti-HEV IgG, and genotype 3 HEV ribonucleic acid (RNA). An acute HEV infection was then considered to be the cause of the hepatitis diagnosed in July 2012. The patient was never given any immunosuppressive therapy, tested negative for HIV, and showed no evidence of hematological or autoimmune disease. Despite ribavirin therapy (600 mg/d), initiated on October 12, 2012, the HEV RNA persisted for 9 months (Fig. 1). Hemoglobin level remained stable under ribavirin treatment (between 12 and 14 g/dL), no anemia were detected. The ribavirin dose was increased to 800 mg/d in June 2013, and plasma HEV RNA was negative in September 2013; it remained undetectable thereafter. His lymphocyte count in June 2013 showed mild T lymphopenia (377 mm^{-3} CD4+ cells and 338 mm^{-3} CD8+ cells; normal value >500 mm^{-3}) but his complement and total IgG concentrations were normal, excluding a common variable immune deficiency. PBMCs were sampled in January 2014, 5 months after virus clearance, and polyclonal stimulation confirmed that his PBMCs were functional (Fig. 2A). His specific IFN-γ response (anti-HEV ELISpot assay) was 23 spot-forming cells (sfc) per 10^6 cells (Fig. 2B). This low concentration of IFN-γ producing cell was in keeping with that found in acutely HEV-infected SOT patients who evolved toward a chronic infection (mean: 9.9 sfc/10^6 cells, Fig. 2B). It was lower than that of acutely HEV-infected IC patients (mean: 149.6 sfc/10^6 cells) or SOT patients who spontaneously cleared the virus (mean: 54.2 sfc/10^6 cells; Fig. 2B). Thus, this patient's chronic HEV infection may be linked to an altered specific IFN-γ T cells response.

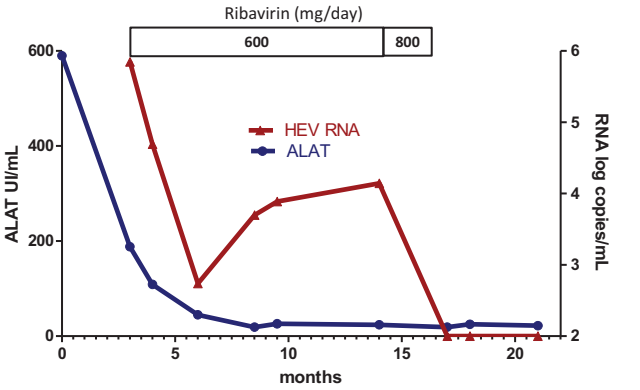

Figure 1. Trend of liver transaminases (ALAT) and hepatitis E virus viremia (HEV RNA) in a chronically infected cirrhotic patient treated with ribavirin. ALAT = alanine aminotransferase, HEV = hepatitis E virus, RNA = ribonucleic acid.

4. Discussion

The key feature of this unusual case of chronic HEV infection is that he had a low IFN-γ specific response to HEV peptides. Nevertheless, his overall T cells responses were apparently functional as anti-CD3/CD28 stimulation was marked by a robust IFN-γ response. The response of this patient was close to that of SOT patients who evolved toward a chronic hepatitis E. We identified no indication of immunosuppression other than a mild lymphopenia in our patient. HEV can evolve toward a chronic infection in HIV-infected patients if the CD4+ T cell count is below 200 mm^{-3}.[4] But, a study reported an HEV infection in a strongly immunosuppressed HIV-positive patient that persisted despite a CD4+ cell count recovery under antiretroviral therapy.[8] Because of the absence of patient's

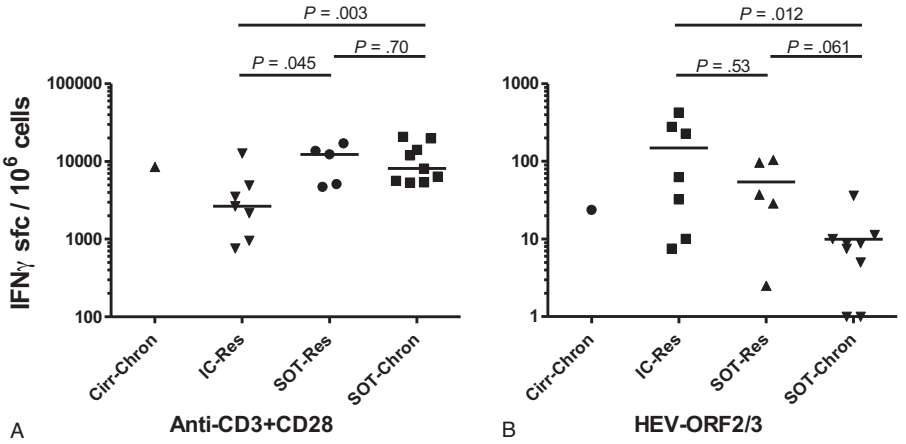

Figure 2. Specific anti-HEV T cells response in SOT patients with a resolutive (SOT-Res, n = 5) or chronic HEV infection (SOT-Chron, n = 9), in our cirrhotic patient with a chronic (Cirr-Chron, n = 1) and in IC patients with a resolutive infection (IC-Res, n = 7). (A) PBMC samples were stimulated with polyclonal stimulation (AntiCD3 +CD28) to assess T cells functionality or (B) with viral peptides (HEV-ORF2/3) and IFN-γ spot-forming cells per 10^6 cells (IFNγ sfc/10^6 cells) were counted by an ELISpot assay. Horizontal bars represent means. ELISpot = enzyme-linked immunospot, HEV = hepatitis E virus, IC = immunocompetent, IFN-γ = interferon γ, PBMC = peripheral blood mononuclear cells, SOT = solid-organ transplant.

blood sample before ribavirin treatment, we were unable to assess if he was transiently immunosuppressed when acute HEV infection occurred. Similar chronic HEV infections are rarely reported in the literature. An HIV-negative patient, who was not given any immunosuppressive therapy, was found to have an undefined CD4+ T-cell defect associated with a chronic HEV infection[9] and a study described a cohort of chronic genotype 4 HEV infection in assumed IC patients[10] but no cell count data were provided. Grewal et al described the case of an IC patient suffering from chronic hepatitis E. He had a history of autoimmune disease and had previously been given immunosuppressive therapy (he had been treated with hydroxychloroquine and steroids nearly 40 years before the hepatitis episode).[11] An undiagnosed immune defect or lymphopenia could also be linked to the chronic evolution in our cirrhotic patient or it could be a diabetes-associated immune dysfunction. This patient probably had underlying NASH cirrhosis although no liver biopsy was performed. The patient cleared the virus with an increased dosage of 800 mg ribavirin; absence of response under 600 mg could be explained by a lack of observance or by a subtherapeutic dosage. Previous in vitro studies on chronic and resolving SOT patients found that chronically infected patients lacked a specific anti-HEV response.[5,6] The patient's persistent HEV infection may be linked to poor production of IFN-γ, a major antiviral cytokine secreted by activated Th1 effectors. Th1 responses are implicated in efficient responses to many viruses and intracellular pathogens and may also be critical for HEV clearance. Although for the present patient, we have investigated the patient T-cell specific response 5 months after virus clearance, in the other groups of patients tested (IC and SOT), the specific responses were measured during the acute phase. However, Brown et al showed that IC HEV-exposed patients had IFN-γ responses still detectable 12 months after the acute phase of infection.[12] In the present case, ribavirin was initiated because he was unable to clear the virus. Ribavirin therapy is now recommended for immunocompromised patients to cure chronic infections.[3] In HEV-infected IC patients, ribavirin could be proposed in specific cases such as patients with severe extrahepatic manifestations[13] or who are unable to spontaneously clear the infection. Other studies are now needed to identify those patients that may evolve toward a chronic infection despite no apparent immunodepression.

References

[1] Kamar N, Dalton HR, Abravanel F, et al. Hepatitis E virus infection. Clin Microbiol Rev 2014;27:116–38.
[2] Kamar N, Rostaing L, Legrand-Abravanel F, et al. How should hepatitis E virus infection be defined in organ-transplant recipients? Am J Transplant 2013;13:1935–6.
[3] Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med 2008;358:811–7.
[4] Dalton HR, Bendall R, Keane F, et al. Persistent carriage of hepatitis E virus in patients with HIV infection. N Engl J Med 2009;361:1025–7.
[5] Suneetha PV, Pischke S, Schlaphoff V, et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. Hepatology 2012;55:695–708.
[6] Abravanel F, Barragué H, Dörr G, et al. Conventional and innate lymphocytes response at the acute phase of HEV infection in transplanted patients. J Infect 2016;72:723–30.
[7] Kamar N, Izopet J, Tripon S, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N Engl J Med 2014;370:1111–20.
[8] Ingiliz P, Mayr C, Obermeier M, et al. Persisting hepatitis E virus infection leading to liver cirrhosis despite recovery of the immune system in an HIV-infected patient. Clin Res Hepatol Gastroenterol 2016;40: e23–5.
[9] Höner zu Siederdissen C, Pischke S, Schlue J, et al. Chronic hepatitis E virus infection beyond transplantation or human immunodeficiency virus infection. Hepatology 2014;60:1112–3.
[10] Liu L, Liu Y, Du Y, et al. Analysis of long-term follow-up and examination of pathological liver tissue in chronic hepatitis E. Dig Liver Dis 2016;48:684–6.
[11] Grewal P, Kamili S, Motamed D. Chronic hepatitis E in an immunocompetent patient: a case report. Hepatology 2014;1:347–8.
[12] Brown A, Halliday JS, Swadling L, et al. Characterization of the specificity, functionality, and durability of host T-cell responses against the full-length hepatitis e virus. Hepatology 2016;64:1934–50.
[13] Blasco Perrin H, Cintas P, Abravanel F, et al. Neurologic disorders in immunocompetent patients with autochthonous acute hepatitis E. Emerg Inf Dis 2015;21:1928–34.