Acute Hepatitis with Severe Cholestasis and Prolonged Clinical Course Due to Hepatitis A Virus Ia and Ib Coinfection

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Background. Acute viral hepatitis due to hepatitis A virus is a self-limited illness that infrequently has a severe clinical course.

Methods. We analyzed the virological characteristics of acute hepatitis A in a patient with a severe clinical presentation (peak total and conjugated bilirubin levels, 65.5 mg/dL and 40.1 mg/dL, respectively) and a course of disease that lasted 7 months.

Results. Hepatitis A virus sequencing revealed coinfection with 2 subgenotypes of hepatitis A virus (Ia and Ib) as etiological factors of the illness.

Conclusions. Hepatitis A virus Ia and Ib coinfection may have accounted for the prolonged and severe course of illness.

Acute viral hepatitis due to hepatitis A virus (HAV) is almost always a mild illness with a benign outcome in babies and children [1]. Although the disease is often symptomatic in adults, a fulminant form occurs infrequently [1, 2]. Also infrequent are other atypical clinical forms of infection, such as relapsing hepatitis, prolonged hepatitis, and cholestatic hepatitis [3, 4]. The virological or host factors involved in the pathogenesis of acute hepatitis A with an atypical course have been poorly investigated, and the association suggested between the severity of liver damage and variability in the 5′ nontranslated region [5, 6] needs further confirmation.

Seven HAV genotypes (I–VII) have been identified [7], 4 of which (I, II, III, and VII) were recovered from humans with HAV infection, and 3 of which (IV–VI) were recovered only from a simian species that developed a hepatitis A–like illness during captivity [7, 8]. Genotypes I and III have both been further subdivided each into 2 distinct groups (subgenotypes Ia and Ib and IIIa and IIIb, respectively), which differ in sequence in ≤15% of nucleotide positions. Genotype I comprises 80% of HAV strains isolated in most countries [9], including Italy. A phylogenetic study of HAV strains performed in 2001 in Puglia, a region of southern Italy where HAV infection is endemic, revealed that genotype I was the only HAV genotype present, with a high prevalence (80%) of subgenotype Ia [10].

We report a case of acute hepatitis A with marked cholestasis and a disease course of >7 months. Both HAV subgenotypes Ia and Ib were identified as etiologic factors of the illness.

METHODS

Patient. A 23-year-old immunocompetent man was admitted to the Infectious Diseases Units of the Second University of Naples in May 2000 who had experienced, over the previous 30 days, symptoms suggestive of severe acute hepatitis. He had marked jaundice (total bilirubin level, 23 mg/dL; direct bilirubin level, 15.6 mg/dL) and a moderate increase in the serum aspartate...
HAV RNA load was determined using sense primer (5′-TGACAGTCACAATCCATCACT-3′ and 5′-TCAACTCCATGATTAGCATG-3′), with the same cycling profile as the first round. Ten μL of the amplified product was analyzed using 2% agarose gel stained with ethidium bromide and detected by hybridization with a specific biotinylated probe in the 5′-NTR region of HAV (5′-GATAGGGTAAACGCGCG-3′), as described elsewhere [4, 12]. In all HAV-RNA positive plasma samples, we determined the HAV load by real-time PCR using a light cycler (LightCycler Hepatitis A Virus Quantification Kit, Roche Molecular System). The detection limit of this method is defined as 200 copies/mL.

The 266 bp region of the VP1/2A junction of the HAV genome was amplified by RT-PCR reaction, as described elsewhere [13]. Primers used in both rounds of PCR were degenerated to enable amplification of all HAV genotypes (universal primers); the primers used in the first round of PCR were sense +2897 5′-CTTTCAGATTGCAAATYTAYAAAT-3′ and antisense −3288 5′-AATTCYATTTCACTGCTCCT-3′, where Y represents C or T. Inner primers were +2949 5′-TATTTGCTTGTCAAGAACAATCAG-3′ (sense) and −3192 5′-AGGRGGTGAAYACTCCATTCTG-3′ (antisense), where R represents A or G.

All HAV RNA-positive samples were sequenced using the BigDye 1.1 terminator kit (Applied Biosystems), following the manufacturer’s instructions, and an ABI 310 automatic sequencer.

Genotyping was performed by sequence analysis after alignment of the outbreak sequences with reference strains using the Bioedit program [14]. The Neighbor-Joining method, implemented using Mega software, version 3.1 [15], with 1000 bootstrap replications, was applied for the phylogenetic analysis.

**Routine methods.** HAV, hepatitis B virus (HBV), and hepatitis D virus (HDV) serum markers were determined by commercial EIAs (Abbott Laboratories assays for anti-HAV IgM, anti-HAV IgG, HBsAg, anti-HBs, total anti-HBc, and anti-HBc IgM and DiaSorin assays for HBeAg, anti-HBe, anti-HDV IgG, and anti-HDV IgM). The anti-HCV antibody was sought by a third-generation commercial EIA (Ortho Diagnostic Systems). Antibodies to HIV-1 and -2 were sought by commercial ELISA (Abbott Laboratories). Liver function tests and markers of autoimmune or metabolic liver diseases were performed according to routine methods. The lymphocyte subsets (e.g., CD4 and CD8) were assessed by flow cytometry using monoclonal antibodies and FACScan (Becton Dickinson).

**CASE REPORT AND RESULTS**

The virologic and clinical courses of infection in the patient are summarized in figure 1. Virologic study of the plasma sam-
amples obtained at the time of hospital admission revealed the presence of HAV RNA (6.7 × 10^5 copies/mL). Because of marked cholestasis (total and direct bilirubin levels, 43 mg/dL and 29 mg/dL, respectively) associated with moderately elevated serum AST and ALT levels (187 IU/mL and 220 IU/mL, respectively), starting on day 42 after the onset of symptoms, the patient was treated with prednisolone (40 mg daily) for 20 days, but no effect on jaundice was observed. In fact, on day 74 after the onset of the symptoms, the total and conjugated serum bilirubin levels reached the highest values (65.5 mg/dL and 40.1 mg/dL, respectively). At that time, we excluded other factors that were possibly involved in the increase of the bilirubin level, such as hemolysis, decreased renal function, and impaired ventilatory function.

A liver biopsy was performed to exclude other possible causes of this severe cholestasis, but the histologic presentation was typical for acute hepatitis with severe cholestasis (i.e., marked cholestasis, with numerous bile thrombi in dilated bile canaliculi encircled by hepatocytes, which were arranged in the form of rosettes, and marked bile stasis in the hepatocytes dominated the histologic presentation). The parenchymal damage was characterized by necrosis with hepatocellular swelling, eosinophilic degeneration, and acidophilic bodies predominantly in zone 3 of the hepatic acini; by foci of cell dropout or intralobular inflammatory cells; and by very small areas of collapse of the reticulin. Active regeneration was determined by the presence of numerous hepatocytes with reduplicated nuclei. Hypertrophy and hyperplasia of the Kupffer cells were also present. Portal tracts were enlarged by moderate infiltration of lymphomonocytic cells. No piecemeal necrosis or fibrosis were observed.

The patient’s clinical condition and liver function improved slowly, and the patient was discharged from the hospital on day 123 after onset. Normalization of the AST and ALT levels was observed by day 132, and normalization of the bilirubin level was observed by day 183. Additional plasma samples were obtained on days 74, 132, and 183. All of these samples yielded positive results by 5’ UTR amplification, and there was a progressive decrease in the viral load (as determined by real-time PCR) for the samples noted on days 74 and 132; the plasma viral load on day 183 was less than the detection limit for the assay, further confirming the tendency toward a clearance of HAV from plasma. On day 218, however, the patient again presented with clinical symptoms, which were prevaently characterized by dyspepsia and moderate jaundice (total and direct bilirubin levels, 3.4 mg/dL and 0.7 mg/dL, respectively) and slightly abnormal serum AST and ALT levels (figure 1). At the time of this mild exacerbation of infection, HAV RNA was detectable (viral load, 4.7 × 10^4 copies/mL). Eighteen days later, the symptoms disappeared, and the findings of liver function tests were normal. The patient remained asymptomatic, with a normal bilirubin level, normal AST and ALT levels, and negative plasma HAV RNA test results for the rest of the 3-year observation period.

Additional virologic tests were performed on the plasma samples obtained from days 35 to 218 to better characterize the HAV strain responsible for the acute hepatitis. All plasma samples but 1 (the sample obtained on day 74) yielded positive results by VP1/2A amplification, which is the region of the hepatitis A genome normally used for phylogenetic studies. The analysis of the nucleotide and amino acid sequences of this region showed the presence of 2 different isolates cocirculating on days 35 and 132 (figure 2) that differed in 17 nucleotide positions in the analyzed region. These nucleotide differences led to 2 amino acid variations. Phylogenetic analysis of the isolates (figure 1) obtained on days 35 and 132 revealed the contemporary presence of 2 different subgenotypes (Ia and Ib); on day 183, only subgenotype Ia was found, whereas on day 218, only subgenotype Ib was identified (figure 3).

DISCUSSION

Our patient had acute hepatitis A, with severe cholestasis, due to both HAV Ia and Ib subgenotypes; the case lasted ~7 months. The 2 subtypes of HAV were acquired contemporaneously, because the patient admitted to only a single time in which he consumed partially cooked shellfish, a risk factor associated with 95% of the cases of hepatitis A in southern Italy [16]. Persistent HAV replication in patients with a prolonged illness has been reported by some authors [4, 17]. Yotsuyanagi et al. [17] described a patient with normalization of AST and ALT levels in 70 days, clearance of HAV RNA from plasma in 60 days, and clearance of HAV RNA from stool in 90 days. Elsewhere, we described 4 patients with normalization of AST and ALT levels in 65–91 days and HAV RNA that was still detectable in plasma specimens 52–94 days after the onset of symptoms [4]. Thus, the prolonged course of illness seems to be associated with delayed clearance of HAV.

Attempts have been made to identify the factors that induce severe courses of hepatitis A. The disease severity has been correlated with host factors, such as the presence of an underlying chronic liver disease [3, 18] or older age of the patient [16]. More recently, the HAV genomes detected in plasma samples obtained from patients with fulminant hepatitis were compared with genomes recovered from patients with self-limiting acute hepatitis, and an association was found between the nucleotide substitutions in the central portion of the 5’ nontranslated region and the severity of the liver damage [5, 6].

No study of the factors inducing a prolonged HAV infection has thus far been published. This first attempt to analyze the HAV genome in a patient with a prolonged course of illness revealed coinfection with HAV strains that were classified as subgenotypes Ia and Ib. This suggests that a coinfection with
Figure 2. Nucleotide alignment (A) and amino acid alignment (B) of the 267 bp region of the VP1/2A junction of the hepatitis A virus genome. Reference strains were GBM (genotype Ia) and HM175 (genotype Ib). Suffixes on the reference strains indicate the genotype. Patient isolates were indicated with the number of days after the onset of symptoms. Suffix B on the patient isolates indicates a second, different isolate from the same sample. dpi, Days postinfection.

2 HAV genotypes or subtypes may be one of the reasons for severe cholestasis and/or a prolonged course of the illness in a patient with hepatitis A. The long-term persistence of HAV infection in our patient may be a consequence of an impairment of the antibody production or of neutralizing activity on the basis of the simultaneous antigenic stimulatory effect of the 2 HAV isolates; this has been previously hypothesized for acute hepatitis C [19]. However, we cannot exclude the possibility that other unidentified factors may influence the course of disease.

Acute and chronic coinfections with other hepatotropic viruses have been frequently reported to be associated with severe forms of acute or chronic liver disease, respectively. The well-known model of acute hepatitis due to HBV/HDV coinfecion is responsible for a high percentage of cases of fulminant hepatitis (3%-7%) in European countries [20] and for outbreaks of fulminant hepatitis in the Amazon basin [21]. Concomitant acute HBV/HCV infection has also been reported to be associated with subfulminant acute hepatitis [22].

In acute hepatitis due to multiple viruses, HDV has a strong inhibitory effect on the HBV genome, and a reciprocal inhibitory effect occurs for HBV/HCV coinfection [21, 22]. Because the viral load for each HAV subgenotype went unexplored in our patient because of the similarity of the 2 genomes and the moderate or low HAV load, the hypothesis of a reciprocal inhibition of Ia and Ib subgenotypes remains to be proven, as does the possibility that such inhibition may be a reason that subgenotype Ib was undetectable on day 183 and again detectable on day 218 when, in turn, genotype Ia had not been isolated. This inhibitory effect has been more clearly demon-
stratified in other hepatitis virus coinfections and has been found to be associated with a severe clinical course [21–24].

It is difficult to understand why a mild exacerbation occurred on day 218, after 35 days of complete remission had occurred. We could venture a hypothesis based on the interaction between the HAV subgenotypes, but there are no published data to support this.

In conclusion, this is, to our knowledge, the first clinical report of HAV sequencing in a patient with acute hepatitis who had severe cholestasis and a prolonged clinical course. Coinfection with the 2 HAV subgenotypes may have accounted for the prolonged and severe course of the illness. However, additional studies are needed to define the host and viral factors responsible for severe hepatitis A.

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References