

## FULMINANT HEPATITIS ASSOCIATED WITH HEPATITIS A VIRUS SUPERINFECTION IN PATIENTS WITH CHRONIC HEPATITIS C

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### ABSTRACT

**Background** Hepatitis A virus (HAV) infection rarely causes fulminant hepatic failure in people with no underlying liver disease. There are limited data on the course of this infection in patients with chronic hepatitis B and chronic hepatitis C.

**Methods** We prospectively followed, from June 1990 to July 1997, 595 adults with biochemical and histologic evidence of chronic hepatitis B (163 patients) or chronic hepatitis C (432 patients) who were seronegative for HAV antibodies. All were tested every four months for serum IgM and IgG antibodies to HAV.

**Results** Twenty-seven patients acquired HAV superinfection, 10 of whom had chronic hepatitis B and 17 of whom had chronic hepatitis C. One of the patients with chronic hepatitis B, who also had cirrhosis, had marked cholestasis (peak serum bilirubin level, 28 mg per deciliter [479  $\mu$ mol per liter]); the other nine had uncomplicated courses of hepatitis A. Fulminant hepatic failure developed in seven of the patients with chronic hepatitis C, all but one of whom died. The other 10 patients with chronic hepatitis C had uncomplicated courses of hepatitis A.

**Conclusions** Although most patients with chronic hepatitis B who acquired HAV infection had an uncomplicated course, patients with chronic hepatitis C had a substantial risk of fulminant hepatitis and death associated with HAV superinfection. Our data suggest that patients with chronic hepatitis C should be vaccinated against hepatitis A. (N Engl J Med 1998;338:286-90.)

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**H**EPATITIS A virus (HAV) infection rarely causes fulminant hepatic failure in people with no underlying chronic liver disease.<sup>1</sup> The outcome of hepatitis A in patients with chronic hepatitis B has been addressed in a few reports.<sup>2</sup> To determine whether the course and the outcome of acute hepatitis A differ between patients with chronic hepatitis C and those with chronic hepatitis B, we prospectively followed, for 86 months, 432 patients with chronic hepatitis C and 163 patients with chronic hepatitis B, testing for serum IgM and IgG antibodies to HAV (anti-HAV) every 4 months. We report the clinical and virologic course and the outcome of acute hepatitis A in 27 patients in whom HAV superinfection occurred.

### METHODS

#### Patients

We prospectively followed, from June 1990 to July 1997, 595 adults (405 men and 190 women; mean [ $\pm$ SD] age, 29.1 $\pm$ 9.8 years) with biochemical and histologic evidence of chronic liver disease related to infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) who were seronegative for anti-HAV at entry into the study. These patients constituted all such patients who had been regularly followed at our institutions for at least 18 months. The characteristics of the patients are summarized in Table 1. Of the 595 patients, 432 patients had antibodies to HCV (anti-HCV) and serum positivity for HCV RNA, and 163 patients had detectable levels of hepatitis B surface antigen (HBsAg) and HBV DNA in serum.

As a control group, we studied 191 consecutive patients with acute hepatitis A who presented during the study period who were negative for both HBsAg and anti-HCV and who had no underlying chronic liver disease. All subjects were tested for serum IgM and IgG anti-HAV every four months. The study was based on regular follow-up of patients with chronic hepatitis B or C. Therefore, specific approval from an institutional review committee or informed consent from the patients was not obtained.

#### Assays

Commercially available enzyme immunoassays (Abbott Laboratories, North Chicago, Ill.) were used to detect IgM and IgG anti-HAV, anti-HCV, HBsAg, antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), IgM and total antibody to hepatitis B core antigen, IgM and total antibody to hepatitis delta (or D) virus (HDV), and IgM antibody to Epstein-Barr virus and cytomegalovirus. Anti-HCV was also detected with a recombinant immunoblot assay (RIBA-3, Ortho Diagnostics, Raritan, N.J.). Antinuclear antibodies and anti-smooth-muscle antibodies were assessed by indirect immunofluorescence on 4- $\mu$ m cryostat sections of rat liver and rat kidney, respectively. The serum samples were considered positive when a fluorescent reaction was detectable in kidney vessel wall alone or in kidney vessel wall, glomeruli, and peritubular structures.<sup>3</sup> Antibodies to asialoglycoprotein receptor were measured by radioimmunoassay.<sup>4</sup>

#### Detection and Quantitation of Viral DNA and RNA

HBV DNA was detected in serum by the polymerase chain reaction (PCR). Briefly, HBV DNA was extracted from 100  $\mu$ l of serum by digestion with proteinase K, followed by extraction with phenol-chloroform.<sup>5</sup> PCR was performed according to a standard protocol with the following primers from the highly conserved HBV surface gene: JOL43 (sense, nucleotides 190 to 217)<sup>6</sup> and

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JOL44 (antisense, nucleotides 787 to 760). The amplified fragment was subjected to agarose-gel electrophoresis and stained with ethidium bromide. Negative controls included normal genomic DNA extracted from serum, leukocytes, and liver and a PCR mixture without template DNA; positive controls were included in each assay and consisted of 100 ng of DNA extracted from HBsAg-positive liver-tumor tissue containing 1 copy of HBV DNA per cell.

Serum HBV DNA was quantified with a chemiluminescent molecular hybridization assay (Digene Diagnostics, Beltsville, Md.) according to the manufacturer's instructions. The sensitivity of this assay is 10 pg of HBV DNA per milliliter of substrate.

HCV RNA was amplified with a nested PCR as described previously.<sup>7</sup> The oligonucleotide primers were located in the 5' untranslated region of the HCV genome: the outer primers were SR1 and SF1, and the inner primers were SR2 and SF2. A quantitative competitive RNA PCR was used to quantify HCV RNA according to the method of Gretch and coworkers<sup>8</sup> after the extraction of total RNA from 100  $\mu$ l of serum according to the method of Chomczynski and Sacchi.<sup>9</sup> Serum HCV RNA was also analyzed (kept frozen at  $-70^{\circ}\text{C}$  and thawed once) by PCR with the Amplicor quantitative PCR system (Roche Molecular Systems, Nutley, N.J.), in which results are expressed as the number of copies of HCV RNA per milliliter.<sup>10</sup> The results obtained by this method were similar to those obtained with the quantitative competitive PCR assay.

HCV genotypes were determined with the Line Probe Assay (INNOLIPA, Innogenetics SA, Ghent, Belgium), which is a reverse hybridization assay based on a highly conserved variation in the 5' untranslated region among the different genotypes. The assay can be used to type and subtype the most common genotypes (1a, 1b, 2a, 2b, 3a, 4, and 5).<sup>11</sup>

Stools were analyzed for HAV RNA by reverse-transcription PCR (RT-PCR). Briefly, RNA was extracted, as described previously,<sup>12</sup> from 400  $\mu$ l of 10 percent stool suspension in phosphate-buffered saline (pH 7.4). Complementary DNA was synthesized with an external antisense primer (A3)<sup>12</sup> in 30  $\mu$ l of reaction mixture containing 200  $\mu$ mol of each deoxynucleoside triphosphate per liter, 1 $\times$  PCR buffer (50 mmol of potassium chloride per liter, 10 mmol of TRIS-hydrochloric acid per liter [pH 8.3], 1.5 mmol of magnesium chloride per liter, and 0.001 percent [wt/vol] gelatin), and 5 U of Moloney murine-leukemia virus reverse transcriptase (Boehringer Mannheim, Mannheim, Germany). After the reverse transcriptase had been inactivated by heating the mixture at  $95^{\circ}\text{C}$  for five minutes, a sense primer (A1)<sup>12</sup> was added to a final concentration of 1.0  $\mu$ mol per liter with 70  $\mu$ l of 1 $\times$  PCR buffer and 2 U of Ampli-Taq (Perkin-Elmer Cetus, Norwalk, Conn.). Amplification was then performed in a thermal cycler for 30 cycles consisting of denaturation for 1 minute at  $94^{\circ}\text{C}$ , annealing for 2 minutes at  $62^{\circ}\text{C}$ , and extension for 1.5 minutes at  $72^{\circ}\text{C}$ .

A second amplification was performed in 100  $\mu$ l of reaction mixture containing 2  $\mu$ l of one of the amplified samples, 1  $\mu$ mol of an internal antisense primer (A2)<sup>12</sup> per liter, 1  $\mu$ mol of the sense primer (A1) per liter, 200  $\mu$ mol of each deoxynucleoside triphosphate per liter, 1 $\times$  PCR buffer, and 2 U of Ampli-Taq under the same conditions as used for the first amplification. Aliquots of the resulting samples were subjected to electrophoresis in 6 percent polyacrylamide gel and stained with ethidium bromide. Water and a sample from a healthy person were used as negative controls. Some of the products of the PCR reaction were separated in 2 percent agarose gel and transferred to a nylon membrane (Schleicher and Schnell, Dassel, Germany). The membrane was then hybridized by Southern blotting with a 19-bp oligonucleotide probe (A6),<sup>12</sup> whose sequence is located between those of the A1 and A2 primers and that was labeled with digoxigenin (Boehringer Mannheim).

Hepatitis G virus (HGV) RNA was detected with a commercial assay (HGV Primer and Capture Probe Set, Boehringer Mannheim).

TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS.\*

CHARACTERISTIC	CHRONIC HEPATITIS B (N = 163)	CHRONIC HEPATITIS C (N = 432)
Sex		
Male	116	289
Female	47	143
Mean age (yr)	30.8	27.5
HBeAg status		
Positive	96	
Negative	67	
HGV infection	16	89
Chronic persistent hepatitis†	57	38
Mean Histological Activity Index‡	2.8	2.9
Mild chronic active hepatitis†	21	349
Cirrhosis	3	152
Prior interferon treatment	0	110
Mean Histological Activity Index‡	7.1	9.2
Chronic active hepatitis†	85	45
Cirrhosis	62	31
Prior interferon treatment	28	9
Mean Histological Activity Index‡	15.4	13.1

\*HBeAg denotes hepatitis B e antigen, and HGV hepatitis G virus.

†The diagnosis was based on histologic findings.

‡The Histological Activity Index is a semiquantitative method of analyzing liver-biopsy findings. Scores can range from 0 (normal level) to 22 (severe chronic active hepatitis or active cirrhosis).

### Liver Biopsy

All 595 patients underwent liver biopsy between June 1989 and May 1990 (i.e., no longer than one year before the beginning of the study). The 20 patients in whom HAV superinfection did not provoke fulminant liver failure had a follow-up liver biopsy one year after recovery, whereas the only patient (of 7) with fulminant hepatitis who survived had a follow-up liver biopsy nine months after recovery. Biopsy findings were scored with the Histologic Activity Index proposed by Knodell and coauthors.<sup>13</sup> In this semiquantitative method, which allows standardization of the interpretation of liver-biopsy findings, the four salient histologic features (periportal or bridging necrosis, or both; parenchymal injury; portal inflammation; and fibrosis) are graded and the scores are totaled. A score of 0 indicates a normal liver; a score of 1 to 3, chronic persistent hepatitis; a score of 4 to 7, mild chronic active hepatitis; a score of 8 to 12, moderate chronic active hepatitis; and a score of 13 to 22, severe chronic active hepatitis or active cirrhosis.

In our departments liver biopsies are performed every five to seven years (according to the judgment of the physician in charge) in all patients with chronic viral hepatitis who give oral informed consent (no written consent is required), in order to follow the evolution of chronic liver disease. Liver biopsies were performed as part of this histologic follow-up in the patients in whom HAV superinfection had occurred, in order to verify whether the ensuing liver damage had caused any variation in the histologic pattern of chronic liver disease. Biopsies were also performed in the five patients with HAV superinfection and cirrhosis to determine whether the disease was still active, since these patients had finally decided to receive interferon treatment, which they had refused before the HAV superinfection and which is indicated only in the presence of active liver disease.

### Statistical Analysis

Differences in proportions among the groups were compared by two-sided Fisher's exact test.<sup>14</sup>

**TABLE 2.** CHARACTERISTICS OF THE PATIENTS WITH ACUTE HEPATITIS A ACCORDING TO WHETHER THEY WERE COINFECTED WITH HBV OR HCV.

CHARACTERISTIC	HBV AND HAV (N=10)	HCV AND HAV (N=17)	HAV ALONE (N=191)
Mean age (yr)	31	28	30
Sex (no.)			
Male	7	10	132
Female	3	7	59
HGV infection (no.)	2	5	8
Chronic persistent hepatitis (no.)	3	3	0
Chronic active hepatitis (no.)	7	14	0
Cirrhosis	2	3	0
Prior interferon treatment	4	3	0
Peak serum ALT (U/liter)*	2439±836	3602±2083	2538±785
Outcome of hepatitis A (no.)			
Uncomplicated course†	9	10	174
Cholestatic course	1	0	17
Fulminant hepatitis	0‡	7	0

\*ALT denotes alanine aminotransferase. The normal value is less than 38 U per liter. Plus-minus values are means ±SD.

†An uncomplicated course was one in which cholestasis or fulminant hepatitis was absent.

‡P=0.03 for the comparison with the other two groups (by Fisher's exact test).

## RESULTS

### Patients with Chronic Hepatitis B and HAV Superinfection

Among the 595 patients with chronic liver disease due to hepatitis B or C, 27 patients acquired HAV superinfection (Table 2), as demonstrated by the presence of IgM anti-HAV in serum and HAV RNA in stool. Ten of these patients (six of whom were HBeAg-positive and four of whom were positive for anti-HBe) had chronic hepatitis B. One patient, who had cirrhosis, had marked cholestasis (peak serum bilirubin, 28 mg per deciliter [479 μmol per liter]); the other nine patients had an uncomplicated course. HBV DNA, which was present before HAV superinfection (mean [±SD], 1710±678 pg per milliliter), became undetectable during the infection in all but 1 patient (in whom it decreased from a level of 2080 pg per milliliter one month before acute hepatitis A to 15 pg per milliliter during the disease) and was again detected (mean, 1260±603 pg per milliliter) in all 10 patients after recovery from acute hepatitis A.

Two of the 10 patients with chronic hepatitis B had an HLA phenotype of A1,B8,DR3, and both also had increased levels of serum gamma globulin (2.0 and 2.1 g per deciliter; normal, 1.8). Antinuclear antibodies were detected in five patients (titer, 1:20 to 1:40), anti-smooth-muscle antibodies in four (1:40), and anti-asialoglycoprotein-receptor antibodies in four (1:100 to 1:200). In none of the patients did HBsAg become undetectable or did the HBeAg or anti-HBe status change. Histologic anal-

ysis did not show any changes one year after recovery from hepatitis A.

### Patients with Chronic Hepatitis C and Uncomplicated HAV Superinfection

Of the 17 patients with acute hepatitis A and chronic hepatitis C, 10 patients had an uncomplicated course of acute hepatitis A (Table 2), 2 of whom were positive for serum HGV RNA both before and during hepatitis A. The peak serum alanine aminotransferase concentration was 2497±648 U per liter. HCV RNA, which was detectable before the HAV superinfection (mean, 10<sup>5</sup> copies per milliliter), became undetectable in 7 of 10 patients during hepatitis A (in the remaining 3 it decreased from 10<sup>5</sup>, 10<sup>6</sup>, and 10<sup>5</sup> copies per milliliter to 10<sup>3</sup> copies per milliliter) and was again detected (mean, 10<sup>4</sup> copies per milliliter) in all 10 patients after recovery from acute hepatitis A.

The HCV genotype was 1b in five patients, 1a in one patient, 2a in three patients, and 3a in 1 patient. None of the patients with uncomplicated hepatitis A had the A1,B8,DR3 HLA phenotype. Antinuclear antibodies were detected in two patients (titer, 1:20), anti-smooth-muscle antibodies in three patients (1:20 to 1:40), and anti-asialoglycoprotein-receptor antibodies in four (1:50 to 1:200). Serum gamma globulin levels did not exceed normal values in any patient. The histologic findings did not show any changes at a follow-up liver biopsy one year after recovery from hepatitis A.

### Patients with Chronic Hepatitis C and Fulminant Hepatitis after HAV Superinfection

Fulminant liver failure developed in seven patients (41 percent) with chronic hepatitis C after HAV superinfection (Table 3). Patients 1, 3, 4, and 7 had traveled to India, Egypt, Morocco, and Thailand, respectively, 32 to 45 days before presenting with fulminant hepatitis. In the case of the remaining three patients, we were unable to identify risk factors for HAV infection. Patient 7 had received 1440 ELISA (enzyme-linked immunosorbent assay) units of inactivated hepatitis A vaccine (Havrix, SmithKline Beecham Biologicals, Rixensart, Belgium) intramuscularly one week before traveling to Thailand. None of the other six patients had received the hepatitis A vaccine. Patients 1 and 4 were former intravenous-drug users; Patients 3, 5, and 7 had acquired HCV through blood transfusions; and Patients 2 and 6 had community-acquired HCV infection. At admission, serum HCV RNA was undetectable in all seven patients, tests for serum IgM anti-HAV and HAV RNA in stool were positive, and tests for IgM antibodies to Epstein-Barr virus, cytomegalovirus, and HDV, as well as for antibodies to hepatitis B core antigen and HBsAg were negative.

Despite intensive supportive therapy, six of the patients died between 8 hours and 12 days after admis-

TABLE 3. CHARACTERISTICS OF SEVEN PATIENTS WITH CHRONIC HEPATITIS C AND HAV-ASSOCIATED FULMINANT HEPATITIS.\*

CHARACTERISTIC	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4	PATIENT 5	PATIENT 6	PATIENT 7
Age (yr)	25	23	32	26	30	31	33
Sex	M	M	F	F	F	M	M
HLA pheno- type	A1,3;B7,8;DR3	A1,2;B8,45;DR3,4	A1,19;B8,40;DR3,5	A2,19;B35,40;DR5,7	A1;B8,17;DR3,7	A3,11;B7,40;DR3	A19;B35,44;DR6,7
HCV genotype	2a	1b	1b	3a	1a	1a	2a
HGV infection	No	No	No	Yes	No	Yes	Yes
Histologic finding	CAH	CAH	CAH	CAH	CAH	CPH	CAH
Prothrombin time (INR)†	4.3	4.1	4.1	5.6	6.2	6.8	5.4
Serum ALT (U/liter)‡	3430	4100	3365	5176	8390	4764	8635
Serum gamma globulin (g/dl)	2.1	2.3	2.4	1.7	3.0	1.2	1.4
Antinuclear antibodies	1:80	1:40	1:80	Negative	1:640	Negative	1:20
Anti-smooth- muscle anti- bodies	1:320	1:320	1:640	Negative	1:640	Negative	Negative
Anti-asialogly- coprotein- receptor antibodies	1:800	1:400	1:1600	Negative	1:800	Negative	Negative
Outcome	Death	Death	Recovery	Death	Death	Death	Death

\*CAH denotes chronic active hepatitis, CPH chronic persistent hepatitis, INR international normalized ratio, and ALT alanine aminotransferase.

†The normal range is 1 to 1.18.

‡The normal value is less than 38 U per liter.

sion. Autopsy showed massive liver-cell necrosis in all six, with a mild-to-moderate infiltration of lymphocytes and plasma cells and (in two patients) neutrophils.

Patient 3 was treated with methylprednisolone (Urbason, Hoechst, Milan, Italy) at an initial dose of 80 mg daily in addition to the usual supportive treatment and regained consciousness within nine days. Methylprednisolone was stopped after three weeks, when autoantibody titers were strikingly reduced (antinuclear antibodies, 1:40; anti-smooth-muscle antibodies, 1:80; and anti-asialoglycoprotein-receptor antibodies, 1:200) and the serum gamma globulin level was 1.9 g per deciliter. Nine months later, tests for serum IgM anti-HAV and fecal HAV RNA were negative; tests for IgG anti-HAV and antinuclear antibodies (titer, 1:20), anti-smooth-muscle antibodies (1:80), and anti-asialoglycoprotein-receptor antibodies (1:50) were positive; and liver biopsy revealed chronic active hepatitis (score on the Histological Activity Index, 9).<sup>13</sup> Six months later HCV RNA was detectable (genotype, 1b; 10<sup>5</sup> copies per milliliter), tests for antinuclear antibodies (titer, 1:20) and anti-smooth-muscle antibodies (1:40) were weakly positive, and tests for anti-asialoglycoprotein-receptor antibodies were negative. In July 1997, 36 months after presentation, tests for antinuclear antibodies and anti-smooth-muscle antibodies were negative.

None of the seven patients with fulminant hepa-

titis were positive for the human immunodeficiency virus, none had used hepatotoxic drugs, and none were alcoholic — in fact, three did not drink alcohol. Four of the patients had the A1,B8,DR3 HLA phenotype, as compared with 9 of the 191 control subjects with acute hepatitis A who were negative for HBsAg and anti-HCV.

## DISCUSSION

HAV infection rarely has a fulminant course and is seldom fatal, with an estimated fatality rate of 0.14 to 2.0 percent.<sup>1,15</sup> The high fatality rate among our patients with chronic hepatitis C and HAV superinfection (35 percent) is thus surprising, as is the even higher percentage of such patients with fulminant hepatitis (41 percent). The seven patients with fulminant hepatitis had chronic hepatitis C but not cirrhosis; thus, the liver parenchyma was still relatively preserved and liver failure was not due to the severity of preexistent liver disease.

Our results confirm previous findings that acute HAV infection does not cause severe liver injury in patients with chronic HBV infection,<sup>16-20</sup> but they differ from the findings of other large series.<sup>2</sup> It is not easy to reconcile such differences; however, in other studies, most HBV-infected patients with fulminant hepatitis A had cirrhosis, the role of HCV was not evaluated, and the possibility of coinfections with HBV and HCV could not be ruled out. Occa-

sionally, acute hepatitis B can lead to fulminant hepatic failure in patients with chronic HCV infection<sup>21</sup>; the latter therefore might be a predisposing condition for acute liver failure after superinfection with other hepatotropic viruses. In our patients, the fact that HCV replication was strikingly reduced during acute HAV infection (a phenomenon of viral interference observed in other patients with both HAV and HBV infections or HAV and HCV infections)<sup>19,22</sup> suggests that HCV does not have a direct role in producing severe liver disease.

HCV infection is associated with autoantibodies, especially anti-smooth-muscle antibodies and antinuclear antibodies; antinuclear antibodies are associated with an HLA phenotype of A1,B8,DR3.<sup>23</sup> In contrast, anti-asialoglycoprotein-receptor antibodies, which are frequently found in HAV infection and at high titers in autoimmune hepatitis (where they may have a pathogenetic role),<sup>24</sup> are almost undetectable in HCV infection.<sup>25,26</sup> In our series, acute HAV infection was associated with a sharp increase in the titers of anti-asialoglycoprotein-receptor antibodies, antinuclear antibodies, and anti-smooth-muscle antibodies and with elevated serum gamma globulin values only in the four patients with the A1,B8,DR3 HLA phenotype and HCV infection, and fulminant hepatic failure developed in all four of these patients. We speculate that acute HAV infection may have triggered autoimmune mechanisms (partly primed by HCV infection) in susceptible patients, which may have increased the liver damage due to hepatitis A and led to massive necrosis of hepatocytes. Infectious hepatitis transmitted by the fecal-oral route (i.e., HAV infection) is linked to the appearance of autoantibodies.<sup>27</sup>

Fulminant hepatic failure developed in three other patients with HCV infection after HAV superinfection without any apparent involvement of autoimmune mechanisms. All three were coinfecting with HGV. A contributory role of preexisting HGV infection in the pathogenesis of fulminant hepatic failure is possible, given the persistence of HGV replication during HAV superinfection, as compared with the inhibition of HCV replication. We cannot, however, establish such a contributory role on the basis of our data. Indeed, the detection of HGV infection in 2 of the 10 patients with chronic hepatitis C in whom hepatitis A had an uncomplicated course suggests that other mechanisms may be important.

HCV and HAV infections are found worldwide; in Western countries hepatitis A is becoming quite rare in children as a result of improved hygiene and now affects mainly adults. Vaccinating patients with chronic liver disease against hepatitis A is recommended in the United States,<sup>28</sup> but not in European countries. Although larger and more prolonged studies are needed, our findings suggest that chronic carriers of HCV who are at risk for HAV infection should be vaccinated against HAV, since superinfection with

this virus may place them at risk for severe, life-threatening acute liver damage.

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