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Precore Mutant Hepatitis B Virus and Outcome of Chronic Infection and Hepatitis in Hepatitis B e Antigen-Positive Children

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ABSTRACT

Mutant hepatitis B virus (HBV), responsible for the lack of hepatitis B virus "e" antigen (HBeAg) secretion because of a translational stop codon at nucleotide 1896 of the HBV-DNA precore region (HBeAg minus HBV), has been detected worldwide in acute and chronic HBV infections and diseases. HBeAg minus HBV appears to condition the outcome of infection and to be involved in the pathogenesis of hepatitis B. We investigated the mutant prevalence and its clinical implications in 30 hepatitis B surface antigen/HBeAg-positive children (17 treated with interferon) with chronic hepatitis B. Wild-type and HBeAg minus HBV were characterized by quantitative oligohybridization assays in sera from 29 children followed up for a mean of 33 mo (12 mo to 9 y). At admission, 18 children (62%) circulated wild-type HBV alone; mutant HBV became detectable in two of them during the follow-up before HBeAg/anti-HBe seroconversion. Wild-type and HBeAg minus HBV were detected in 10 children (34.5%); mutant HBV levels were lower than 20% of total viremia in four of them and higher in six. Serum HBV-DNA from one child did not hybridize with our probes. HBeAg minus HBV was associated with older age ($p < 0.009$) and higher histologic activity ($p < 0.069$). HBeAg/anti-HBe seroconversion occurred independently from HBeAg minus HBV detection; it was observed in six (37.5%) of 16 children with wild-type HBV alone and in four (33.3%) of 12 children with mixed viremia. In cases with mixed viremia, seroconversion occurred in four (66%) of six with HBeAg minus levels lower than 20% of total viremia but in none of six with higher levels ($p < 0.066$). These findings suggest that HBeAg minus HBV can be detected in chronic HBV carrier children; it has relevant clinical implications and affects the outcome of both HBV infection and disease when its relative prevalence reaches values higher than 20% of total viremia. (***Pediatr Res* 36: 347–350, 1994**)

Abbreviations

HBV, hepatitis B virus
anti-HBe, antibody to HBeAg
HBeAg, hepatitis B virus "e" antigen
HBsAg, hepatitis B surface antigen
ALT, alanine aminotransferase
IgM anti-HBc, antibodies against hepatitis B core antigen of class M
IFN, interferon
gen, genome

HBV mutants unable to secrete HBeAg have been isolated from both acute and chronic hepatitis B patients worldwide (1–4). The mutation accounting for more than 95% of HBeAg-defective HBV so far described is a G-A switch at nucleotide 1896 that creates a translational stop codon (1–6). Such a mutant (HBeAg minus HBV) appears to be an important pathogenetic determinant of HBV infection in adult patients (7). Mother-to-child transmission of mixed populations of wild-type and HBeAg minus HBV is associated with acute self-limiting hepatitis and HBV clearance in newborns (8, 9). Perinatal infection with wild-type HBV alone often results in chronic HBV carriership (9, 10). High levels of HBV viremia in the absence of liver damage are common in HBsAg/HBeAg-positive children (11); nevertheless, about two thirds of them clear HBV replication before adulthood with annual HBeAg/anti-HBe seroconversion rates of 10–19% (12, 13). Seroconversion is usually associated with hepatitis exacerbation (12). In adults, HBeAg to anti-HBe seroconversion follows the loss of immuno-

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tolerance and the onset of the HBV immunoelimination phase, which were found to be associated occasionally with the *ex novo* HBeAg minus HBV detection (3, 14, 15). However, when mutant HBV reaches levels higher than 20%, it is associated with viral replication persistence (14).

To investigate the relations between HBeAg minus HBV and the outcome of chronic hepatitis B, we characterized quantitatively the circulating viral populations of 30 HBsAg/HBeAg-positive children, followed up for a mean of 33 mo.

METHODS

Patients. We included in the study 30 consecutive HBsAg-positive children with evidence of chronic HBV infection (lasting for more than 6 mo). They were followed up at the Pediatric Clinic of the University of Torino for a mean of 33 mo (range 12 mo to 9 y) with blood controls obtained at least every 3 mo. Their median age was 8 y (ranging from 2 to 14 y); 15 were males and 15 females. Twenty-seven of them had persistently elevated serum ALT levels (mean value 133 U/L, range 51–801 U/L) and detectable serum IgM anti-HBc and HBV-DNA; the remaining three were asymptomatic with normal liver biochemistry despite viremia levels higher than 10^7 gen/Eq/mL. None of them were anti–hepatitis C virus, anti–hepatitis D virus, and anti-HIV positive. Seventeen patients were treated with IFN according to the protocol of a controlled multicenter randomized trial (16). Monthly visits were conducted during hepatitis exacerbation or during IFN treatment.

The potential sources of HBV infections were perinatal transmission from HBsAg/HBeAg-positive mothers in seven cases, household contact in 17, blood product in one, and unknown in two. Three adopted children were born in geographic areas hyperendemic for HBV infection, and a perinatal transmission cannot be ruled out.

Liver histology. A liver biopsy was available from 16 patients: the histologic diagnoses were consistent with minimal lesions in one patient, chronic persistent hepatitis in 12, and mild chronic active hepatitis in the remaining three.

Assays. Routine blood chemistry tests including a liver function test (AST, ALT, γ-glutamyltranspeptidase, alkaline phosphatase, serum albumin, gammaglobulins, total bilirubin, and prothrombin time) were done using standard procedures. HBV serologic markers were detected by RIA (AUSRIA II, CORAB, HBe kit, Abbott Laboratories, North Chicago, IL) using 10 Paul Ehrlich Institute units as positive/negative cut-off [0.200 IMx index value (IMx is a microparticle enzymatic immunoassay) (16)]. HBV-DNA was detected semiquantitatively in whole serum (150 μL) by a dot-blot hybridization assay (17). The overall sensitivity of the test was about 10^5 gen/Eq/mL. Negative specimens were amplified by polymerase chain reaction using a commercial amplification kit (Cetus Corp., Emeryville, CA) and primers encompassing the precore and core region. The primer sequences were 5′-GGGGAGGAGATTAGGTTAA-3′ (1744–1761) and 5′-GGCAAAAACGAGAGTAACTC-3′ (1940–1959), respectively (15). Thirty cycles of amplification were carried out in a thermal cycler (Perkin Elmer Cetus, Emeryville, CA): 95°C for 2 min (5 min for the 1st cycle), 55°C for 2 min, 72°C for 2 min, and final extension at 72°C for 10 min. Amplification products were detected by ethidium bromide staining with an overall sensitivity of 10^4 gen. Wild-type and HBeAg minus HBV were characterized in 104 sera after PCR amplification by an oligohybridization assay as previously reported (14). Amplified DNA (10 μL) was spotted on nitrocellulose filters, incubated with ^{32}P 5′ end-labeled oligonucleotides without mutations (5′-TGGCTTTGGGGCATGGAC-3′) or with two nucleotide mutations (5′-TGGCTTTAGGACATGGAC-3′) under stringent hybridization and washing conditions, and autoradiographed as reported (14). The sensitivity, specificity, and reproducibility of the assays were controlled by repeated tests and end-point dilution curves of wild-type and HBeAg minus reference sera (14).

Statistical analysis. Statistical analysis was performed by using χ^2, Mann-Whitney U, Fisher's, and Spearman's correlation tests.

RESULTS

At admission, viremia was detectable by dot-blot hybridization in 24 (80%) of 30 HBsAg/HBeAg-positive children. The mean HBV-DNA level was 10^6 gen/Eq/mL; the levels ranged between 10^7 and 10^8 gen/Eq/mL in five cases and between 10^6 and 10^7 gen/Eq/mL in five cases and were 10^6 gen/Eq/mL in 13 cases and 10^5 gen/Eq/mL in one case. After PCR amplification, serum HBV-DNA became detectable in five of the remaining six patients; it remained undetectable in one child (3.3%) who seroconverted from HBeAg to anti-HBe during 5 mo follow-up. Circulating viral populations were studied in 29 children: wild-type HBV alone was detected in 18 patients (62%), and mixed viral populations of both wild-type and HBeAg minus HBV were found in 10 (34.5%). HBV-DNA amplified from the serum of one child did not hybridize with our probes. HBeAg minus HBV levels were lower than 20% of total viremia in four (40%) of 10 children with mixed viral populations and ranged between 20 and 40% in the remaining six. Baseline serum levels of viral replication (HBV-DNA), virus-induced liver disease (IgM anti-HBc) and liver cell damage (ALT), histology, median age, and sex of children with exclusive wild-type or mixed HBV populations are shown in Table 1. Children with detectable amounts of HBeAg minus HBV were older than children with exclusive wild-type HBV populations (median age was 10 *versus* 5 y, $p = 0.009$, $z = 2.601$, Mann-Whitney U test).

During the follow-up period, HBeAg/anti-HBe seroconversions, associated with the clearance of serum HBV-DNA and persistent ALT serum level normaliza-

Table 1. *Virologic, clinical and demographic features of 28 children according to circulating HBV population**

	Viral population	
	Homogeneous wild-type HBV	Mixed
Number of patients	18 (10)†	10 (7)†
HBV-DNA (gen/Eq/mL)		
Median value	10^7	10^6‡
Range	10^4–10^8	10^4–10^8§
IgM anti-HBc		
>0.200 IMx	13/18	6/8‖
ALT		
Median value	88 U/L	83 U/L‡
Range	23–801	27–245
ML	1	
CPH	9	3¶
CAH		3
Median age	5 y	10 y
Range	2–14	7–14
Male/female	8/10	5/5

* IMx, microparticle enzymatic immunoassay; ML, minimal lesions; CPH, chronic persistent hepatitis; CAH, chronic active hepatitis

† Number of children treated with IFN in parentheses.

‡ Any significant difference was found in HBV-DNA levels and ALT values among children with HBeAg minus levels lower or higher than 20% of total viremia.

§ $p = 0.035$, $z = 2.104$, Mann-Whitney U test.

‖ Serum for IgM anti-HBc detection was not available in two patients.

¶ $p = 0.069$.

tion, occurred in 11 (36.6%) of 30 children, eight of them treated with IFN. The loss of serologic markers of viral replication was observed in eight (44.4%) of 18 children with exclusive baseline wild-type viremia. HBeAg minus HBV became detectable in two of them at the time of the hepatitis flare-ups (ALT levels increased >2 SD from the baseline value) that preceded their HBeAg/anti-HBe seroconversion. In one case, HBeAg minus HBV reached a 20% relative prevalence; nevertheless, in this child both wild-type and mutant viruses were cleared at the end of a liver cell necrosis episode that lasted about 2 mo. Ten mo later, the patient recovered with HBsAg/anti-HBs seroconversion; this was the only case with complete resolution of HBV infection among 30 children (Fig. 1). HBeAg to anti-HBe seroconversion and clearance of viral replication occurred in two (50%) of four patients with baseline levels of HBeAg minus HBV lower than 20% of total viremia. Instead, serum HBV-DNA remained detectable in six patients with baseline mutant virus levels higher than 20% of total viremia. In one of them, HBeAg minus HBV levels increased during the follow-up: a major increment of the relative prevalence of HBeAg minus HBV (a shift from 30 to 50% of total viremia) was observed after a mild liver cell necrosis episode (ALT levels of 140 U/L) that was not associated with changes in the HBeAg/anti-HBe status.

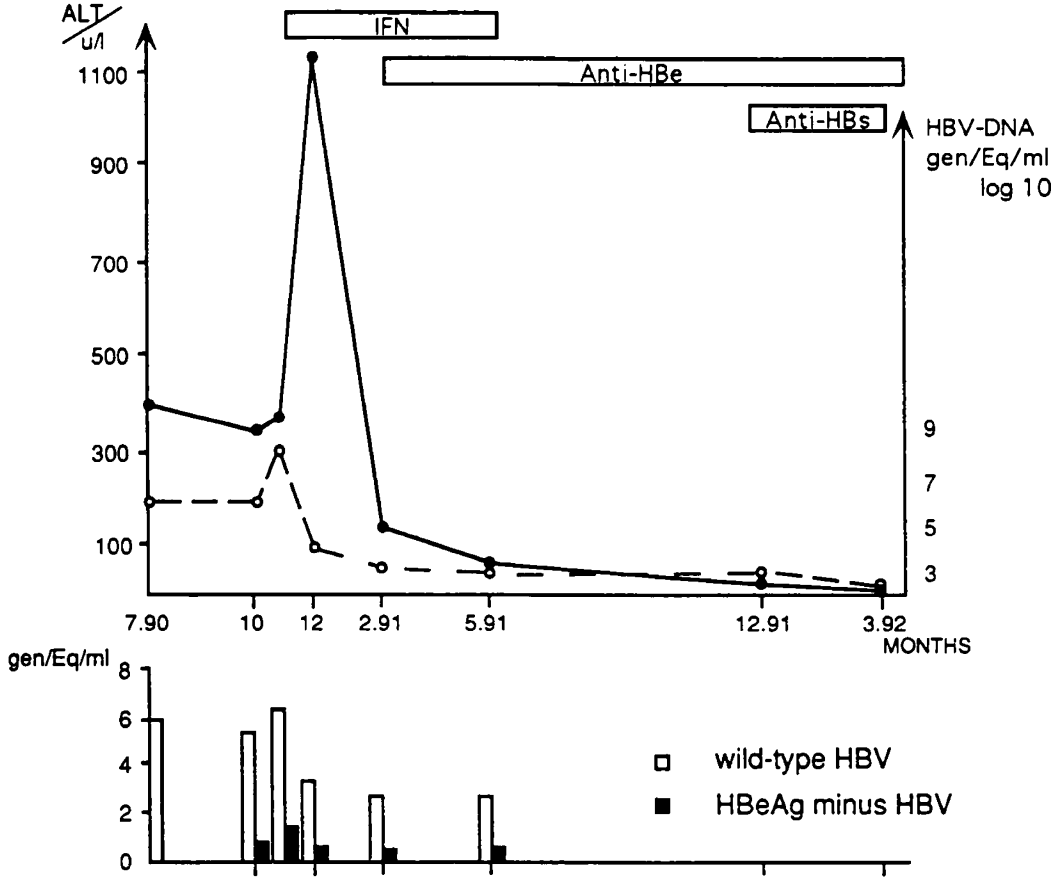

Figure 1. Outcome of chronic hepatitis in a 5-y-old child who seroconverted from HBeAg during IFN therapy and to anti-HBs 7 mo thereafter. He had wild-type HBV alone at baseline. HBeAg minus HBV became detectable before starting IFN treatment, and it reached its highest serum levels at the peak of the ALT flare-up.

Overall, we found HBeAg minus HBV (at admission or during the follow-up) in 12 patients. HBeAg to anti-HBe seroconversion occurred in 66% (4/6) of children with mutant HBV serum levels lower than 20% or with *ex novo* HBeAg minus detection during the follow-up but in none of the children with higher levels of HBeAg minus viremia ($p < 0.066$).

DISCUSSION

Chronic HBV infection sustained by both wild-type and HBeAg minus HBV appears to be a frequent finding in HBeAg-positive children as well as in adults with chronic hepatitis B. We found that the proportion of HBeAg-positive children with mixed infections is comparable to that observed in adults (36.8%) (14). One third of HBsAg/HBeAg-positive children (10 of 29, 34.4%) circulated mutant HBV as a minor viral population at admission, and two additional patients developed detectable levels of HBeAg minus HBV during the follow-up. Apparently the presence (<20% of total viremia) of HBeAg minus HBV did not affect the rate of either spontaneous or IFN-induced HBeAg/anti-HBe seroconversion compared with that observed in adults. *Ex novo* HBV mutant detection during hepatitis flares in two patients with homogeneous baseline wild-type HBV viremia was associated with an efficient virus immunoelimination and virus replication clearance leading to anti-HBe seroconversion and recovery from hepatitis. In contrast, viral replication and liver disease persisted in all children (three of them treated with IFN) with baseline HBeAg minus HBV levels higher than 20% of overall viremia. In these patients, HBeAg minus HBV appears to have warranted virus persistence under immunopressure, and furthermore, inefficacious immunoelimination attempts determined the mutant virus progressive selection. The HBeAg minus HBV ability to escape the immunoelimination is confirmed by its higher relative prevalence after the hepatitis flare-ups (15).

Prevalence (21.4%) of HBeAg-positive children with elevated mutant HBV levels appears to be slightly higher than that of HBeAg-positive adults (7.8%) (13). This could be explained by lower immunocompetence of young children compared with adults with a consequent longer-lasting inefficient immunoelimination phase that selects HBeAg minus HBV. A less competent immunosystem favoring virus persistence and HBeAg minus HBV selection could determine transition of chronic hepatitis B from the HBeAg to the anti-HBe form that prevails in adults. Prospective studies of cohorts of HBeAg-positive children, after anti-HBe seroconversion, can answer this question.

In conclusion, the results of the study support the view that HBeAg minus HBV plays a relevant role in the pathogenetic mechanisms of chronic HBV infection. Precore heterogeneity of HBV-DNA appears to have relevant implications and to condition the outcome of both HBV infection and disease in both children and adults. *Ex novo* HBeAg minus HBV detection is associated with the beginning of the immunoelimination phase, but the inefficiency of clearing the virus can result in mutant selection. When HBeAg minus serum levels reach values higher than 20% of total viremia, the rates of spontaneous and IFN-induced recoveries appear significantly reduced.

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