Long-Term Follow-up of Chronic Hepatitis B Virus Infection in Children of Different Ethnic Origins

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The natural history of chronic hepatitis B in children is influenced by mode of transmission and varies with regional endemicity. Seroconversion rates were studied in 174 hepatitis B e antigen (HBeAg)–positive children who were of different ethnic origins and living in Canada. Overall, 40.2% became anti-HBeAg positive, and 8.6% were hepatitis B surface-antigen positive during a mean follow-up of 4.5 years. Spontaneous seroconversion rates were lower in Asian-born, mainly vertically infected, children, versus those born either in Canada or where horizontal transmission predominates (24% vs. 44%, $P = .015$). Kaplan-Meier analysis showed that the cumulative persistence of HBeAg after 13 years was 25% in Asian-born children, versus 6% in all others ($P < .05$). Treatment of 27 children accelerated seroconversion by 3 years, without influencing the proportion seroconverting over time. Thus, although Asian-born children seroconvert more slowly, a large proportion will seroconvert before adulthood. Because treatment appears to accelerate anti-HBe seroconversion, longitudinal studies are required in order to assess the long-term benefits of early treatment.

Universal vaccination against hepatitis B virus (HBV) infection was introduced into Taiwan in 1984 but not into most industrialized nations until 1991 [1]. The result has been a significant reduction in perinatal and horizontal transmission of HBV and in the incidence of hepatocellular carcinoma (HCC) [2, 3]. In the United States, a region considered to be of low endemicity, despite a reduction of ~60% in the incidence of hepatitis B during 1984–1994, an estimated 185,000 new infections still occur each year [4]. Furthermore, the increased incidence of HCC in the United States is thought to be caused by the increasing incidence of hepatitis C virus (HCV) infection and by increased immigration from regions where HBV is endemic. Infections that occurred during the 1960s and 1970s now are reaching 2–3 decades of evolution [5]. Published estimates of the risk for development of HCC in HBV carriers vary widely but are generally highest in Asian countries and Alaska (240–494/100,000 carrier-years) and lowest in western countries (0–13/100,000 carrier-years) [6]. A clear understanding of the natural history of HBV infection would permit appropriate intervention to prevent the development of complications.

The natural history of chronic HBV infection in children has been determined from limited data that vary by geographic region. In a Chinese study, only 7% of children cleared hepatitis B e antigen (HBeAg) during a mean follow-up of 4 years, and all children remained hepatitis B surface-antigen (HBsAg) positive [7]. In contrast, Evans et al. [8] found a higher seroconversion rate (13%) in 454 HBeAg-positive Asian-American carriers (47% were children), who were followed a median of 25 months [8]. Of these patients, 95% were born in Asia but live in the United States.

In a long-term follow-up study by Bortolotti et al. [9], 185 Mediterranean children were followed an average of 13 years (some were treated). Of these children, 84% cleared HBeAg, whereas only 6% lost HBsAg. Five children developed liver cirrhosis, which progressed to HCC in 2 of them [9]. A minority (14%) of these children were born to mothers who tested positive for HBsAg; similar long-term studies for children infected at birth are lacking.

Here we describe the long-term outcome of chronic HBV infection in a heterogeneous group of children of different ethnic origins. All lived in a similar environment in the Province of Quebec, Canada.

Patients and Methods

Patients. Over 20 years (1980–2000), 174 children who were 1 month–18 years (mean, 4.7 ± 4.6 years) old and had hepatitis B infection were consecutively observed. They were followed for a median of 4.9 ± 3.1 years (range, 4 weeks–16 years) at the Department of Pediatric Gastroenterology and Hepatology of Hôpital Sainte-Justine in Montreal. Approximately two-thirds of the patients were referred from the International Adoption Clinic at Hôpital Sainte-Justine, because of positive results of HBV serology screening. This clinic, in operation since 1989, sees about 50% of the 1000 children from foreign countries adopted in Quebec each year. The remaining patients were referred for evaluation of elevated aminotransferases

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Table 1. Demographic features of hepatitis B virus–infected children at presentation, by birth region.

	Total	Asian	Eastern European	Canadian	Other
Patients, no.	174	75	21	34	44
Age at diagnosis, mean years ± SD	4.7 ± 4.6	2.03 ± 1.25	4.2 ± 3.3	5.8 ± 4.4	7.86 ± 5.7
Boys/girls, no.	72/102	15/60[a]	14/7	20/14	23/21
Adopted, no.	97	67	19	2	9
Follow-up, mean years ± SD	4.5 ± 3.1	4.2 ± 2.8	4.6 ± 2.3	5.7 ± 4.1	3.9 ± 3.0
Transmission, no. (%)					
HBsAg-positive mother	34 (19.5)	5 (6.7)	2 (9.5)	16 (47.1)	11 (25)
Transfusion	5 (2.9)	1 (1.3)	0	3 (8.8)	1 (2.3)
Family contact	15 (8.6)	1 (1.3)	2 (9.5)	5 (14.7)	7 (15.9)
Percutaneous exposure	2 (1.2)	1 (1.3)	0	1 (2.9)	0
Unknown	118 (67.8)	67 (89.3)[b]	17 (81.0)[b]	9 (26.5)	25 (56.8)
Other diseases, no. (%)					
Glomerulonephritis	1 (0.6)	1 (1.3)	0	0	0
HCV coinfection	8 (4.6)	2 (2.7)	1 (4.8)	2 (5.9)	3 (6.8)

NOTE. HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.
[a] $P < .05$, Asians vs. all others.
[b] These children were infected during the first year of life either by vertical transmission or by contact in foster homes or families.

and positive results of HBV serology. All children were HBsAg positive. The presence of chronic hepatitis was confirmed, on the basis of liver histology, in 42 patients (23.2%).

The patients were divided into 4 groups, on the basis of region of birth. The Asian group included children born in China and Vietnam. The eastern-European group comprised children born in Romania and Russia. The Canadian children were all born to parents born in Canada; no native Canadian children were followed. The fourth group (others) comprised children born in Haiti, Pakistan, Bangladesh, India, and Latin America.

Of 174 children, 27 (15.5%) were treated within the context of clinical trials. The first trial, with interferon (IFN)–α, recruited patients during 1992–1994. Inclusion criteria were as follows: HBsAg positive for ⩾6 months, HBV DNA- and HBeAg-positive twice, alanine aminotransferase (ALT) levels >2 times normal, and chronic hepatitis on liver biopsy [10]. The second trial recruited patients during 1999–2000, for lamivudine therapy. These children were 2–17 years old; were HBsAg positive, HBV DNA positive, and HBeAg positive; and had ALT levels >1.3 times normal [11]. All patients who met the entry criteria during each period participated in the study. A response to therapy was defined as a loss of HBeAg, development of anti-HBe, clearance of HBV DNA from serum, and normalization of ALT levels. During follow-up, patients were usually seen in our outpatient clinic every 3–12 months, for physical examination and biochemical and serologic testing. Serum samples were obtained and stored at −20°C. Beginning in 1992,

Table 2. Alanine aminotransferase (ALT), hepatitis B e antigen (HBeAg), hepatitis B surface antigen (HBsAg), hepatitis B virus (HBV) DNA, and α-fetoprotein levels in hepatitis B virus–infected children at study entry, by birth region.

	Total	Asian	Eastern European	Canadian	Other
Patients, no. (%)	174	75 (43.1)	21 (12.1)	34 (19.5)	44 (25.3)
ALT, no. (%)					
Normal	57 (32.8)	28 (37.3)	7 (33.3)	5 (14.7)[a]	17 (38.6)
<2× normal	31 (17.8)	17 (22.7)	0[b]	9 (26.5)	5 (11.4)
>2× normal	70 (40.2)	26 (34.7)	11 (52.4)[b]	16 (47.1)	17 (38.6)
>10× normal	16 (9.2)	4 (5.3)	3 (14.3)	4 (11.8)	5 (11.4)
HBeAg positive, no. (%)	168 (96.6)	73 (97.3)	20 (95.2)	32 (94.1)	43 (97.7)
Anti-HBe positive, no. (%)	6 (3.4)	2 (2.7)	1 (4.8)	2 (5.9)	1 (2.3)
HBsAg positive, no. (%)	173 (99.4)	74 (98.7)	21 (100)	34 (100)	44 (100)
Anti-HBs positive, no. (%)	1 (0.6)	1 (1.3)	0	0	0
HBV DNA tested, no. (%)					
Positive (>5 pg/mL)	93 (83.8)	45 (83.3)	9 (75)	17 (85)	22 (88)
Negative	18 (16.2)	9 (16.7)	3 (25)	3 (15)	3 (12)
Total	111	54	12	20	25
α-Fetoprotein, no. (%)					
Normal (0.0–9.1 μg/L)	147 (94.2)	68 (95.8)	16 (84.2)	28 (100)	35 (92.1)
Abnormal (>9.1 μg/L)	9 (5.8)	3 (4.2)	3 (15.8)	0	3 (7.9)
Total	156	71	19	28	38

NOTE. Anti-HBe, hepatitis B e antibody; anti-HBs, hepatitis B surface antibody.
[a] Canadians vs. all others.
[b] $P < .05$, eastern Europeans vs. all others.

Table 3. Histopathologic analysis of liver-biopsy samples of hepatitis B virus–infected children, by birth region.

	Total	Asian	Eastern European	Canadian	Other
Biopsy samples , no. (%)	41	7 (17.1)	5 (12.2)	19 (46.3)	10 (24.4)
Histology, no. (%) of biopsy samples					
Minimal hepatitis	8 (19.5)	3 (42.9)	2 (40)	2 (10.5)	1 (10)
Mild hepatitis	25 (61)	2 (28.6)	3 (60)	13 (68.4)	7 (70)
Moderate hepatitis	7 (17.1)	2 (28.6)	0	4 (21.1)	1 (10)
Severe hepatitis	1 (2.4)	0	0	0	1 (10)
Fibrosis, no. (%) of biopsy samples					
None	17 (41.5)	5 (71.4)	3 (60)	5 (26.3)	4 (40)
Mild	20 (48.8)	1 (14.3)	1 (20)	14 (73.7)	4 (40)
Moderate	3 (7.3)	0	1 (20)	0	2 (20)
Cirrhosis, no. (%) of biopsy samples	1 (2.4)	1 (14.3)	0	0	0

all patients were monitored, with yearly abdominal ultrasound and serum α-fetoprotein testing.

Methods. HBsAg; HBeAg; antibodies to HBe, HBs, and hepatitis D; and α-fetoprotein levels were measured by commercial immunoassay kits (Abbott Laboratories). HBV DNA was analyzed semiquantitatively by dot-blot hybridization, beginning in 1989 (assay cutoff point, 5 pg/mL). In 27 of 41 patients, liver biopsy was performed, as per study protocols, prior to treatment; in the rest, it was performed either because of persistently elevated aminotransferases or to confirm the suspicion of cirrhosis. The biopsy was performed with a Jamshidi needle under sedation and local anesthesia, by a percutaneous intercostal or subcostal approach.

Statistical analysis.—For statistical analysis of data, we used the Pearson χ^2 test. We compared differences between groups by use of unpaired Student t tests, adjusted for the inequality of variances when within-group variances were statistically different according to the Brown-Forsythe test. For continuous variables, we used the Mann-Whitney nonparametric test. $P < .05$ was considered statistically significant.

For preliminary examination of the survival distribution across groups, we used Kaplan-Meier survival curves, which were compared by log-rank test. Since patients were recruited into the study at various ages, we then used regression survival analysis based on the proportional-hazards model (Cox regression survival analysis), to evaluate the potential impact of the predictors, controlling for the age at diagnosis. Finally, we used multivariate Cox regression survival analysis with stepwise selection of explanatory predictors, to extract the best subset of predictors.

Results

Demographics. Table 1 lists the demographic features of the 174 children at presentation to our clinic. Most of the children from Asia and eastern Europe were adopted as young infants. The Asian children tended to have been adopted at a younger age, although the difference from the other groups was not statistically significant. The higher proportion of girls in the study population was mainly due to the large number of girls in the Asian group, a phenomenon directly related to the adoption policies in the countries of origin. The mode of transmission was classified as unknown when clear documentation was unavailable. Because of the high adoption rates in the Asian and eastern-European groups, the mode of transmission for the majority of children in these groups was not reliably documented. However, most Chinese and Vietnamese infants have been infected by vertical transmission, whereas eastern-

Table 4. Hepatitis B e antigen (HBeAg) seroconversion in hepatitis B virus–infected children, by birth region.

	Total	Asian	Eastern European	Canadian	Other
Patients, no.	174	75	21	34	44
Untreated patients, no.	147	70	17	22	38
Treated patients, no.	27	5	4	12	6
Seroconversions, no.					
Spontaneous					
HBeAg to HBeAb	51 (34.7)	17 (24.3)[a]	9 (52.9)	10 (45.5)	15 (39.5)
HBsAg to HBsAb	11 (7.5)	6 (8.6)	2 (11.8)	2 (9.1)	1 (2.6)
Treatment induced					
HBeAg to HBeAb	19 (70.4)	4 (80)	3 (75)	7 (58.3)	5 (83.3)
HBsAg to HBsAb	4 (14.8)	1 (20)	2 (50)	1 (8.3)	0
Total seroconversions, no. (%) of total treated					
HBeAg to HBeAb	70 (40.2)	21 (28)[b]	12 (57.1)	17 (50)	20 (45.5)
HBsAg to HBsAb	15 (8.6)	7 (9.3)	4 (19.0)	3 (8.8)	1 (2.3)

NOTE. Ab, antibody; HBs, hepatitis B surface.

[a] $P = .036$, Asians vs. eastern Europeans.

[b] $P < .05$, Asians vs. all other groups.

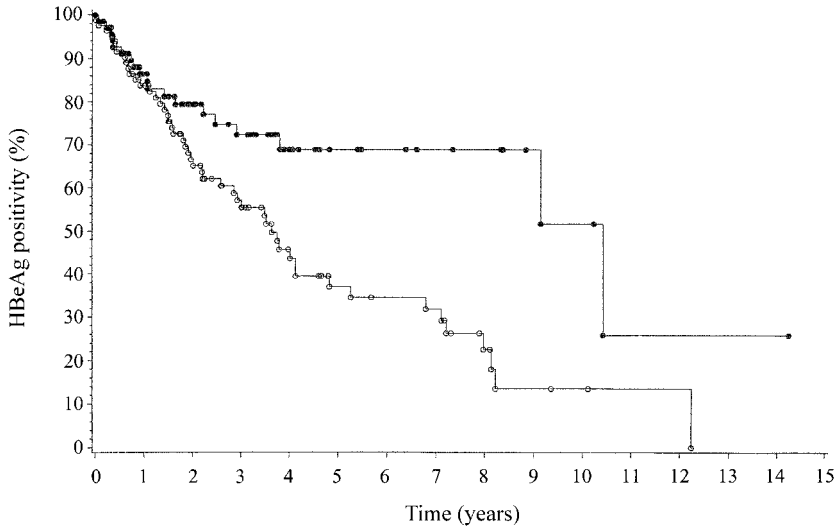

Figure 1. Cumulative proportion of children from different ethnic groups maintaining hepatitis B e antigen (HBeAg) positivity during follow-up as calculated by the Kaplan-Meier test in the total series of HBeAg-positive cases and adjusted for age at diagnosis. $P = .01$, Asian vs. all other groups. ●, Asian; ○, all other groups.

European adoptees tend to have been infected during the first year of life.

All children were asymptomatic at presentation and remained so throughout follow-up. Eight patients, distributed evenly among the groups, were coinfected with HCV. No patient was coinfected with delta virus. One patient had glomerulonephritis. No child presented with HCC, nor did any develop HCC during the follow-up period.

Of the children studied, 96% were HBeAg positive at entry into the study; however, the 6 HBeAg-negative children were found to be HBeAg positive in another medical center (table 2). Elevated (>2 times normal) serum ALT levels at presentation were less common in the Asian group (39.9%) than in the other groups (eastern Europe, 66.7%, $P = .04$; Canadian, 58.8%, not significant [NS]; other, 50%, NS). Of the 123 patients in whom HBV DNA testing was performed, 75% had detectable levels; no difference was observed among groups. Serum α-fetoprotein testing was done in 90% of patients, and 90% of these had normal levels. In the 9 patients with abnormal (>9.1 μg/L) serum α-fetoprotein concentrations, the levels were only marginally above normal, and all became normal during the first 6 months of observation, regardless of seroconversion status.

Liver biopsy was done in 41 children, in most cases to determine their eligibility for treatment within clinical trials (table 3). Compared with the other groups, more patients in the Canadian group were biopsied, mainly because of the higher proportion of children with abnormal (85.3%, vs. 63%, 66.6%, and 61.4%) serum ALT levels. Results of studies of liver histology showed minimal or mild inflammation in 80.5% of biopsy samples and mild or no fibrosis in 90.3% of biopsy samples. Only 1 Asian child had histologic evidence of cirrhosis.

Twenty-seven children (15%) were treated with either IFN-α ($n = 19$) or lamivudine ($n = 8$). Only 6.6% of Asian children were treated, compared with 35% of Canadian children, partly because of their lower aminotransferase levels and partly because of our policy of not treating children with IFN who are <2 years old.

HBeAg seroconversion. HBeAg seroconversion was observed in 70 (40.2%; table 4) of the children. Of 147 untreated children, 51 (34.7%) seroconverted to anti-HBe–positive status, compared with 19 (70.4%) of the treated children; 12 patients seroconverted during therapy, and 7 seroconverted a mean of 14 months after therapy (range, 2–30 months). Asian children showed significantly lower spontaneous seroconversion rates than did the eastern-European children. When only children with ALT levels >2 times normal were considered, there was no seroconversion-rate differences between groups.

Figure 1 and figure 2 demonstrate the product-limit estimates of the proportion of subjects who remained HBeAg positive during follow-up, expressed in terms of time after diagnosis. Asian children (figure 1) seroconverted significantly more slowly than did all other children. By 13 years after diagnosis, 75% of Asian children, compared with 94% of all others, had seroconverted ($P < .05$). Although treated children showed a tendency to seroconvert faster, there was no statistical difference between the two groups (figure 2). In this study, 75% of untreated children, versus 86% of treated children, had HBeAg seroconversion by 13 years after diagnosis.

The mean ALT level at presentation did not differ between children who showed spontaneous seroconversion (244 ± 397 IU/L) and those who showed treatment-induced seroconversion (282 ± 444 IU/L). However, children who did not seroconvert

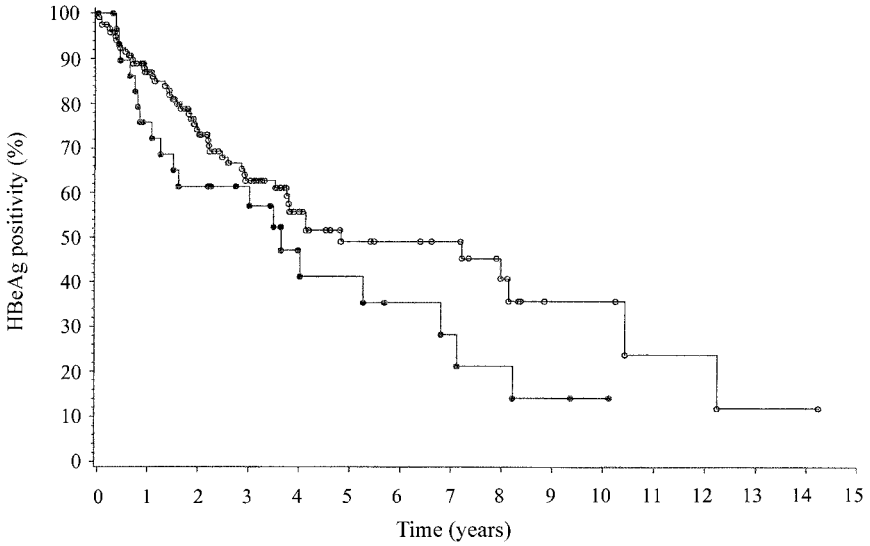

Figure 2. Cumulative proportion of children maintaining hepatitis B e antigen (HBeAg) positivity during follow-up as calculated by the Kaplan-Meier test in the total series of HBeAg-positive cases, in patients treated with either interferon or lamivudine (●) and in untreated patients (○). No statistically significant difference was seen ($P = .12$).

presented with significantly lower ALT levels (44 ± 41 IU/L; $P = .0001$). The mean HBV DNA level was not significantly different between the groups. The mean time to normalization of ALT (5.8 ± 2.5 months) in untreated patients who seroconverted paralleled the time to disappearance of HBV DNA (5.38 ± 3.4 months) and was not significantly different from the mean time to normalization of ALT in treated patients (4.4 months).

HBsAg seroconversion. HBsAg seroconversion was observed in 15 patients (8.6%). Treatment was associated with seroconversion in 14.8% of children, whereas spontaneous seroconversion occurred in 7.5%. The mean time to HBsAg seroconversion in treated children was 31 months, and that in untreated children was 4.2 ± 3.1 years.

Figure 3 shows the cumulative proportion of HBsAg persistence during follow-up, both in untreated children and in children treated with IFN-α or lamivudine. We found no statistically significant difference in percentage of HBsAg seroconversion, on the basis of either ethnic group or sex.

After follow-up for 1–8 years, children with proven vertically transmitted disease seemed to seroconvert HBsAg more slowly than did children with other modes of transmission. However, because of the small size of the sample, the results were not statistically significant (data not shown).

Discussion

This report has described the long-term outcome of chronic hepatitis B in a heterogeneous group of children of different ethnic origins who lived in the Province of Quebec. We observed a survival rate of 100%, and no subject developed either liver failure or signs of portal hypertension during the follow-up period of ⩽16 years. Of the children with chronic HBV infection, 90% had normalized ALT levels before reaching adulthood. These data are similar to those that Bortolotti et al. [12] reported in children of Mediterranean origin who had been infected mainly by horizontal transmission.

Two main transmission patterns with geographic implications have been described in the literature. Perinatal infection predominates in Asia, where most HBsAg-positive mothers have circulating HBeAg, whereas in Africa, eastern Europe, and the Mediterranean basin, where the proportion of HBeAg-positive women is much lower, infection is transmitted during infancy and childhood, mainly by chronically infected family members and playmates [13, 14] and by therapeutic injections via improperly sterilized needles [15–17]. Thus, although the mode of transmission in many of the Asian-born children in the present study was not documented, it can be reasonably assumed that the disease in most of these children has been transmitted perinatally. This hypothesis is strengthened by our observation that Asian children had lower aminotransferase levels and seroconverted anti-HBe significantly more slowly than did all other patients, particularly those from eastern Europe. Perinatal infection is associated with prolonged immune tolerance to HBV and is characterized by an absence of symptoms, normal or slightly elevated ALT activity, HBeAg positivity, and high levels of circulating HBV DNA [7].

Although the Asian-born children seroconverted to anti-HBe more slowly than did all other children, the spontaneous-seroconversion rate was 24% during the follow-up period, and the cumulative seroconversion to anti-HBe by 13 years after diagnosis was as high as 75%. These data contrast with a se-

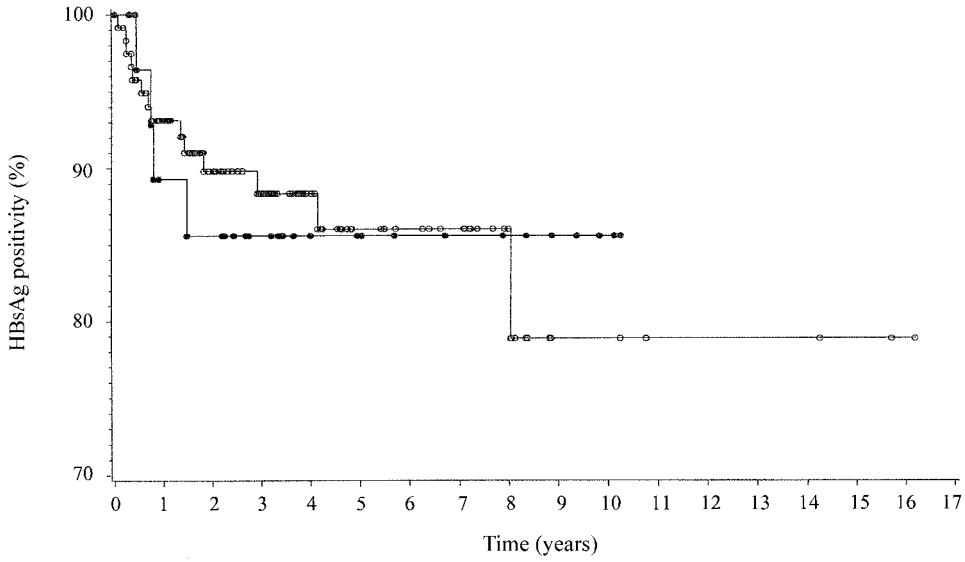

Figure 3. Cumulative proportion of children maintaining hepatitis B surface antigen (HBsAg) positivity during follow-up as calculated by the Kaplan-Meier test in the total series of HBsAg-positive cases, in patients treated with either interferon or lamivudine (●), and in untreated patients (○) ($P = .9$).

roconversion rate of only 7% over 4 years in Asians born and living in Asia [7] but are comparable to those reported by Evans et al. [18], who found that 13% of Asians living in the United States seroconverted to anti-HBe during a median follow-up period of 25 months and who predicted that >90% of HBV carriers infected at birth would become HBeAg negative by age 50 years. Such differences in long-term outcome in patients with the same ethnic origin but living in different countries have been described for other aspects of hepatitis B. Thus, although long-term studies of Asian individuals suggest a high risk of HCC development among patients infected at birth, Villeneuve et al. [6] reported a very low risk of development of HCC in individuals living in the Montreal area, even among Asian patients. These differences might be attributed to environmental influences on either the host or the virus.

One possible environmental factor influencing the immune response to HBV is the nutritional status of the child. We observed that most Asian children adopted by Canadian families were initially malnourished but improved rapidly. There may also be a role for viral coinfection. For example, in Asian countries, the high population density might favor the transmission of herpesviruses, including cytomegalovirus and human herpesvirus 6, which are common and have immunosuppressive properties. Possibly, infection by such agents at an early age may contribute to the prolonged immunotolerant phase of hepatitis B infection. Finally, the susceptibility to HCC in HBV-infected patients may be influenced by dietary aflatoxin exposure, which is particularly common in Asia.

The natural history of hepatitis B was observed in the 147 patients who were not treated. There was no statistically significant difference in the cumulative proportion of treated children who seroconverted, compared with untreated children. However, there was an acceleration of anti-HBe seroconversion of at most 3 years in treated patients (figure 2); the population that we studied comprises a large proportion of chronic hepatitis B–infected children who were likely to have been vertically infected. Similar findings have been reported in children infected mainly by horizontal transmission [19, 20].

These data, along with those of other pediatric studies in which no difference, in the long-term HBeAg seroconversion rate, between treated and untreated children was observed [12, 19–21], raise important questions about the benefits and timing of therapy in children with chronic hepatitis B. It remains to be shown whether acceleration of HBeAg seroconversion influences the development of complications, such as liver cirrhosis or HCC. Liver cirrhosis was observed in only 1 patient and was an early complication, rather than the end point of long-lasting liver disease. Cirrhosis in children with hepatitis B usually affects young boys and follows early biochemical remission and clearance of HBeAg [22–24]. Only longitudinal studies of liver histology can determine whether early treatment confers long-term benefits.

Biochemical remission of hepatitis was associated with loss of serum HBV DNA in most patients, independent of ethnic origin and type of treatment. However, in 8.5% of children who seroconverted to anti-HBe, HBV DNA remained elevated, even though the semiquantitative analysis was by dot-blot hybridization, which is less sensitive than polymerase chain reaction

(PCR). It has been observed that, with the use of the more sensitive PCR technique, a minimal level of HBV replication may persist in at least half of children for ⩾10 years after anti-HBe seroconversion and biochemical remission [21]. The long-term implication of this finding remains unclear.

Twenty years after the availability of hepatitis B vaccine, we are still far from worldwide eradication of the disease. Until universal vaccination becomes a reality, the long-term complications of HBV disease—particularly cirrhosis and HCC—remain significant problems. Although early treatment with either lamivudine or IFN seems to be an attractive approach to alteration of the course of chronic hepatitis B, the benefits of universal therapy remain to be proven. Children with elevated aminotransferases seroconvert spontaneously, in a period of time that is similar that seen for children receiving treatment. Overall, treatment affords only an acceleration of 2–3 years in HBeAg seroconversion and only a slight advantage in HBsAg seroconversion. Long-term follow-up of children in randomized clinical trials would answer this central question more reliably than would analysis of heterogeneous populations. The ultimate impact of a 3-year acceleration in seroconversion may be important but, as yet, remains unknown.

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