INFECTION AND IMMUNITY, July 1983, p. 83–87
0019-9567/83/070083-05$02.00/0

Vol. 41, No. 1

Hepatitis B Vaccine: Further Studies in Children with Previously Acquired Hepatitis B Surface Antigenemia

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Received 10 February 1983/Accepted 8 April 1983

Three doses of inactivated hepatitis B vaccine were given at 1-month intervals to 31 hepatitis B surface antigen (HBsAg)-positive Senegalese children aged between 3 and 24 months. A control group of 18 HBsAg-positive Senegalese children received diphtheria-tetanus-polio vaccine. Immunization of HBsAg-positive infants with hepatitis B vaccine was safe but inefficient. After a 12-month follow-up, the prevalence of HBsAg chronic carriers was not significantly reduced in the hepatitis B vaccine group as compared with the control group: 48.4 and 66.7%, respectively. The presence of hepatitis B antigen was found to be a major risk factor for HBsAg-positive children to develop a chronic carrier state. The risk of developing an HBsAg chronic carrier state was also related to advancing age at time of enrollment in the study.

In Western countries (Europe, North America) the prevalence of hepatitis B (HB) is low in the general population, and the disease is mainly confined to high-risk populations such as hemodialyzed or polytransfused patients, hospital and laboratory staff, and male homosexuals. Since 1975, a vaccine against HB (HB vaccine) was developed in France and applied to the prophylaxis of this disease in humans (18). The safety, potency, and efficacy of the HB vaccine was clearly assessed in high-risk settings in France (13, 19). Similar results have been obtained in high-risk population trials (10, 11, 21). In contrast to the situation in Europe or the United States, HB infection is endemic in Southeast Asia and tropical Africa, where close to 100% of the adult population is or has been infected by HB virus (HBV). In such areas, chronic HB surface antigen (HBsAg) carriers may represent 10 to 20% of the population. Carriers are at a high risk of developing chronic liver disease leading to cirrhosis and ultimately to primary hepatocellular carcinoma (6, 17). Epidemiological data show that, for most of the cases, the HBsAg carrier state is a result of HBV infections acquired very early in life (7, 22).

A controlled clinical trial of the HB vaccine in children was set up in Senegal to prevent such early infections. Senegal is a country where HBV infection is endemic (15% HBsAg in the adult population) and the incidence of primary hepatocellular carcinoma is high (30 to 40 cases per 100,000 per year). The program "Prevention Hépatite-Hépatome" was conducted in a rural area of Senegal, the Niakhar district, located in the center of the peanut basin (16). Before the immunization program, an epidemiological study showed that early HBV infections resulted in a rate of 17% HBsAg carriers at the age of 24 months (5). The study assessed that the HB vaccine was effective in preventing early HBV infections occurring in children within their first 2 years and thus reducing the carrier rate by 85% (13, 15). The immunization program was intended to decrease the incidence of the HBsAg carrier state in children, and at first, we planned to immunize only HBsAg-negative children. However, for psychological and practical reasons, this protocol could not be achieved. First, mothers would not have understood the exclusion of some children, and second, it was not possible to perform on the field a sensitive HBsAg screening before immunization. Therefore, every child aged less than 2 years was included in the program irrespective of his HBV status. It happened that some children were already infected by HBV when they enrolled in the trial.

The purpose of the following work was to investigate the effect of HB vaccine immunization in children with previously acquired HBs antigenemia. HBsAg clearing in immunized children was compared with a control group of HBsAg-positive children who did not receive

TABLE 1. Sex and age distribution of study groups

Parameter	HB vaccine group (n = 31)		Control group (n = 18)	
	No.	%	No.	%
Age (mo)				
3–6	7	22.6	3	16.7
7–12	6	19.4	4	22.2
13–24	18	58.0	11	61.1
Sex				
Males	15	48.4	8	44.4
Females	16	51.6	10	55.6

HB vaccine. The anti-HBs response of children who cleared their HBsAg is also reported.

MATERIALS AND METHODS

Study Populations. A total of 49 HBsAg-positive children entered this study: 31 were immunized with the HB vaccine (HB vaccine group) and 18 received a diphtheria-tetanus-polio (DTP) vaccine (control group). Children were aged between 3 and 24 months when they received the first dose of either vaccine. The two groups were similar in age and sex distribution (Table 1). All children studied herein were followed for at least 12 months after the first injection.

The efficacy of HB vaccine was assessed by comparison of the HBsAg clearing and anti-HBs seroconversion rate in both groups.

HB vaccine. The immunizing antigen was purified from HBsAg-positive sera of HB antigen (HBeAg)-negative asymptomatic carriers (3). One dose of vaccine (1 ml) contained 5μg of purified HBsAg of both subtypes *ad* and *ay*. The vaccine was inactived with Formalin, and aluminium hydroxide was added as adjuvant at a concentration of 0.1%. The HB vaccine was produced, according to our method, by Institut Pasteur Production, Paris (1). It received its sale license under the name HEVAC B.

DTP vaccine. The control group received a DTP vaccine, IPAD-DTP, obtained from Institut Pasteur Production, Paris.

Protocol and follow-up. Only healthy children were involved in the study, with their parents being fully informed of the purposes and modalities of the trial. Children of the HB vaccine group received three injections of HB vaccine (1 ml subcutaneously) at 1-month intervals and a booster injection after 1 year. Children of the control group were given DTP vaccine according to the same schedule. Children of the HB vaccine group received the DTP vaccine sometime after the third injection of the HB vaccine; at least 2 months later.

Blood samples were taken on the day of the first injection of vaccine (T_0), the day of the third injection (T_2), 2 months after the third injection (T_4), and at the time of the booster injection (T_{12}). Blood samples were taken 2 years after the first injection (T_{24}) in five children of the HB vaccine group and in three children of the control group.

Laboratory methods. HBV markers were detected by radioimmunoassays: HBsAg by AUSRIA-II, anti-HBs by AUSAB, anti-HB core (HBc) by CORAB, and HBeAg and anti-HBe by Abbott-HBe (all kits from Abbott Laboratories, North Chicago, Ill.).

Anti-HBs positivity was expressed as the ratio of the net count of the sample to the mean net count of negative controls (P/N ratio), with a cutoff value of 2.1. Anti-HBs titer was estimated by using a semiquantitative procedure recommended by the manufacturer in which an estimated radioimmunoassay unit value was determined by calculating the ratio of the reactivity of the unknown specimen to the positive control: S/P ratio = [sample (counts per minute) − negative control (counts per minute)]/[positive control (counts per minute) − negative control (counts per minute)]. From the S/P ratio, the anti-HBs titer could also be expressed in milli-international units per milliliter (up to a ratio of 1.6), with a standard curve established by Hollinger et al. (14).

Serum alanine aminotransferase could not be measured in field conditions owing to the lability of the enzyme.

RESULTS

As previously reported, neither local nor general reactions were observed after immunization with HB vaccine, even in HBsAg-positive children. Mothers reported that none of the injections was followed by significant side effects such as vomiting, fever, fatigue, or other pain.

Clearing of HBsAg. At the end of the follow-up (T_{12}), 15 children (48.4%) of the HB vaccine group and 12 (66.7%) of the control group were still HBsAg positive (χ^2 = 1.538; not significant). Among these children, five of the HB vaccine group and three of the control group had a blood sampling 2 years after the beginning of the protocol: all of them were still HBsAg carriers.

TABLE 2. HBV marker status of children at T_0

HBV marker status				HB vaccine group (n = 31)		Control group (n = 18)	
HBsAg	HBeAg	Anti-HBe	Anti-HBc	No.	%	No.	%
+	+	−	+	15	48.4	8	44.4
+	−	−	+	2	6.5	1	5.6
+	−	−	−	9	29.0	4	22.2
+	ND[a]	ND	+	5	16.1	5	27.8

[a] ND, Not dc

TABLE 3. Twelve-month evaluation of initially HBeAg-positive children

Age at T_0 (mo)	Group (no.)	HBV status at T_{12}					
		HBsAg + HBeAg +		HBsAg + HBeAg −		Anti-HBs+ Anti-HBc+	
		No.	%	No.	%	No.	%
≤12	HB vaccine (4)	2	50.0	0		2	50.0
	Control (3)	2	66.7	0		1	33.3
13–24	HB vaccine (11)	10	90.9	0		1	9.1
	Control (5)	4	80.0	1	20.0	0	
Total	HB vaccine (15)	12	80.0	0		3	20.0
	Control (8)	6	75.0	1	12.5	1	12.5

The efficacy of HB vaccine was further studied as follows.

(i) HBV marker status of the children before immunization. The HB vaccine group and the control group were similar in relation to HBV marker status before immunization (Table 2). The prevalent HBV marker profile was represented by HBeAg-positive children: 15 children in the HB vaccine group (48.4%) and 8 in the control group (44.4%). A second important group was made of children with HBsAg alone (HBeAg and anti-HBe negative, anti-HBc negative): nine children (29.0%) in the HB vaccine group and four (22.2%) in the control group. HBeAg and anti-HBe antibody were not tested for five children of the HB vaccine group and five children of the control group because of a lack of serum.

(a) HBsAg clearing among HBeAg-positive children. At the end of the follow-up (T_{12}), 12 children (80.0%) from the HB vaccine group and 7 (87.5%) from the control group were still HBsAg positive (Table 3; Fig. 1).

(b) HBsAg clearing among HBeAg-negative children. At T_{12}, only 1 of the 11 children (9.1%) who received the HB vaccine was still HBsAg positive compared with only 1 of the 5 children (20.0%) in the control group as well (Table 4; Fig. 1). A total of 72.7% (8 of 11) of the vaccinated children were anti-HBs positive at the time of the booster injection, of whom 2 had associated anti-HBc antibody. One vaccinated child was found to be seronegative at T_{12}, although he had responded to the third injection of HB vaccine; in this case anti-HBs had disappeared between T_4 and T_{12}. One vaccinated child did not respond to the vaccine but remained anti-HBc alone at T_{12}. Anti-HBs detection could have possibly been missed by the timing of serum collection. None of the four children of the control group who cleared HBs antigenemia developed anti-HBs: three were found to be seronegative and one had anti-HBc alone at T_{12}.

(ii) Age. The relative risk of developing an HBsAg chronic carrier state according to age is presented in Table 5. In the HB vaccine group, 3 of 13 infants (23.1%) aged less than 12 months remained HBsAg carriers against 12 of 18 children (66.7%) aged 13 to 24 months ($P < 0.02$). In the control group, 2 of 7 infants (28.6%) aged less than 12 months remained HBsAg carriers against 10 of 11 children (90.9%) aged 13 to 24 months ($P < 0.03$). No prognostic value could be assigned to HBe antigenemia according to age due to the small number of children in each subgroup.

Anti-HBs humoral immune response to HB vaccine in children who cleared HBsAg. Mean anti-HBs titers in children who seroconverted after HB vaccination were 32 mIU/ml after two injections of HB vaccine, 111 mIU/ml after three injections, and 75 mIU/ml at the time of the booster injection.

DISCUSSION

The HB vaccine was ineffective in eliminating HBsAg among children who had already ac-

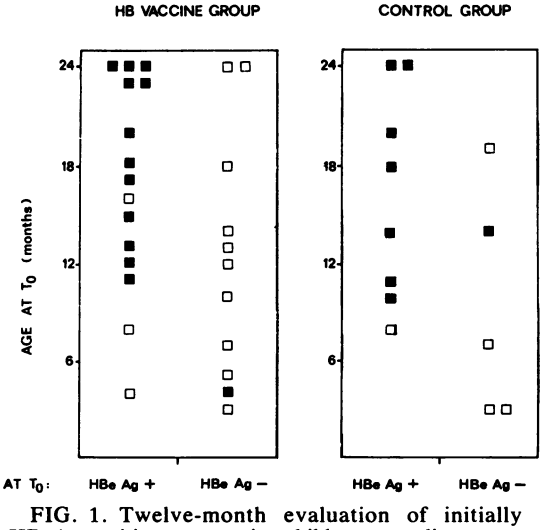

FIG. 1. Twelve-month evaluation of initially HBeAg-positive or -negative children according to age at T_0. Symbols: ■, HBsAg positive at T_{12}; □, HBsAg negative at T_{12}.

TABLE 4. Twelve-month evaluation of initially HBeAg-negative children

Group (no.)	HBV status at T_{12}									
	HBsAg positive		Anti-HBc alone		Anti-HBs alone		Anti-HBs+ Anti-HBc+		Seronegative	
	No.	%	No.	%	No.	%	No.	%	No.	%
HB vaccine (11)	1	9.1	1	9.1	6	54.5	2	18.2	1[a]	9.1
Control (5)	1	20.0	1	20.0	0		0		3	60.0

[a] Responded to the third injection of HB vaccine.

quired HBs antigenemia at the time of the first injection of vaccine. After a 12-month follow-up, the prevalence of HBsAg chronic carriers was not significantly reduced in the HB vaccine group compared with the control group: 48.4 and 66.7%, respectively. This is in agreement with a recent report by Dienstag et al. in a study including 16 American HBsAg carriers who received six monthly doses of Merck HB vaccine (12).

The presence of HBeAg was found to be a major risk factor for HBsAg-positive children in developing a chronic carrier state. A total of 80% of the HBeAg-positive children in the HB vaccine group became chronic carriers against 9.1% of the HBeAg-negative children ($P < 0.001$). Similarly, in the control group, 87.5% of the HBeAg-positive children became chronic carriers against 20.0% of the HBeAg-negative children ($P < 0.05$). This is in agreement with the prognostic value frequently attributed to HBeAg (2, 20). The risk of developing an HBsAg chronic carrier state was also related to advancing age at time of enrollment in the study (Fig. 1). It was then possible to grade the relative risk for an HBsAg-positive child to develop a chronic carrier state. Children at lower risk were the HBeAg-negative children, then came the HBeAg-positive children aged less than 12 months, and ultimately the HBeAg-positive children aged 13 to 24 months (Fig. 2).

Mean anti-HBs titers obtained in HBsAg-positive children who seroconverted after HB vaccination were lower than titers reported previously in seronegative children (4, 15). Mean anti-HBs titers in seroconverted children were 32 mIU/ml after two injections of HB vaccine, 111 mIU/ml after three injections, and 75 mIU/ml at the time of the booster injection, compared with 57, 268, and 162 mIU/ml, respectively, in immunized seronegative children.

Of the 49 HBsAg-positive children who entered this study, 13 (26.5%) were anti-HBc negative (9 in the HB vaccine group and 4 in the control group). They were also HBeAg and anti-HBe negative. Eight of them (61.5%) were aged less than 1 year. It is difficult to explain the high prevalence of this HBV marker status. These children could have been at the very early stage of HBV infection, i.e., before the appearance of anti-HBc. Anti-HBs was not detected at T_{12} in the four children of the control group, whereas eight of nine children of the HB vaccine group seroconverted to anti-HBs. Among the four children of the control group, three were seronegative at T_{12} and only one remained an HBsAg carrier. Anti-HBs response could have been underrated by the timing of serum sampling, lack of a low immune response, and a rapid

TABLE 5. Risk of becoming an HBsAg chronic carrier according to age

Age at T_0 (mo)	HB vaccine group		Control group	
	No. tested	No. of chronic carriers (%)	No. tested	No. of chronic carriers (%)
≤6	7	1 (14.3)	3	0
7–12	6	2 (33.3)	4	2 (50.0)
Total				
≤12	13	3 (23.1)	7	2 (28.6)
13–24	18	12 (66.7)	11	10 (90.9)

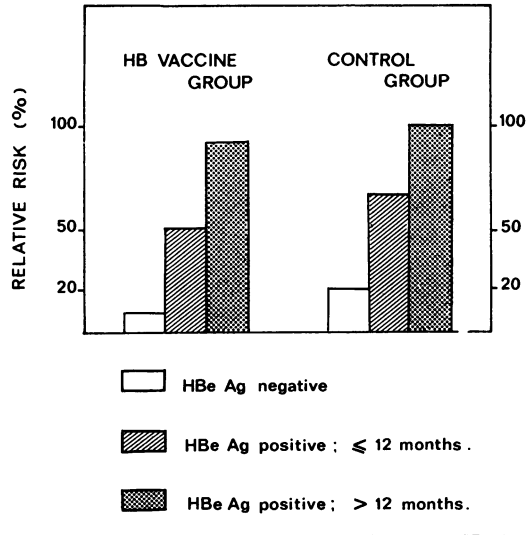

FIG. 2. Relative risk of becoming an HBsAg chronic carrier according to HBeAg positivity and age at T_0. Symbols: □, HBeAg negative; ▨, HBeAg positive, ≤12 months; ▩, HBeAg positive, >12 months.

decrease in antibodies. It is also possible that these three children were infected at a low level without a serological HBV marker. In such cases, molecular hybridization with cloned HBV DNA as a probe might be useful in detecting persistent HBV infection in subjects who are negative for all known serological HBV markers (8, 9).

The safety of HB vaccine in HBsAg-positive children would permit the immunization of entire young populations without serological prescreening during mass immunization campaigns in endemic areas. However, results of the present work stress the necessity of immunizing children before acquisition of HBsAg in endemic areas such as Senegal since 66.7% of HBsAg-positive children aged 13 to 24 months develop a chronic carrier state despite immunization. To be fully effective, a program of prevention should therefore focus on very early immunization. The administration of the first dose of HB vaccine within the first hours of life would be optimal since a satisfactory anti-HBs immune response has been obtained in neonates (4, 15).

ACKNOWLEDGMENTS

This work was inspired and directed by Philippe Maupas until his death on 6 February 1981.

The Franco-Senegalese program "Prevention Hépatite-Hépatome" was supported by grant 28/77/432, project 223/DH/77, from the Ministère de la Coopération (France) and the Secrétariat d'Etat à la Recherche Scientifique et Technique (Senegal).

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