

HUMORAL IMMUNE RESPONSE FOLLOWING HEPATITIS B VACCINE BOOSTER DOSE IN CHILDREN WITH AND WITHOUT PRIOR IMMUNIZATION

Voranush Chongsrisawat¹, Apiradee Theamboonlers¹, Sawan Khwanjaipanich², Somchai Owatanapanich³, Supakarn Sinlaparatsamee⁴ and Yong Poovorawan¹

¹Viral Hepatitis Research Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and Hospital, Bangkok 10330, Thailand; ²Chonburi Provincial Hospital, Chon Buri 20000, Thailand; ³Pediatric Department, Lopburi Hospital, Lop Buri Province 15000, Thailand; ⁴Maharat Nakhon Si Thammarat Hospital, Nakhon Si Thammarat 80000, Thailand

Abstract. One hundred and twenty-three children who had received no, incomplete and complete primary hepatitis B vaccination but had negative or very low anti-HBs titer were immunized with a single dose of recombinant hepatitis B vaccine. Blood tests for anti-HBs were obtained at 30 ± 5 days after the booster immunization. Twelve of 18 (66.7%) children without prior immunization (group 1) seroconverted following the single dose. Seroconversion rates in children who had undetectable anti-HBs with incomplete and complete primary immunization (group 2 and 3) were 83.34% and 94.5%, respectively. All children with complete 3-dose vaccination but who had low anti-HBs titer (group 4) also seroconverted. This study confirmed that immunological memory, allowing a protective anamnestic response, lasted at least 8 years in children who had received primary HB immunization with undetectable anti-HBs. Therefore, we conclude that the booster dose after complete vaccination is not necessary in healthy children.

INTRODUCTION

Persistent hepatitis B virus (HBV) infection may lead to the development of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. It is estimated that there are 300 million HBV carriers and as many as 1 million deaths annually due to HBV infection worldwide (Treadwell *et al*, 1993). Various therapies for persistent HBV infection, such as interferon- α or antiviral agents, have been developed but the goal of eradicating HBV globally depends largely on efforts to prevent new infection. In an attempt to reduce the global impact of HBV infection, in 1991 the WHO recommended that HB vaccine should be integrated into national immunization programs by the year 1997 (WHO, 1991). Thailand has included HB vaccines into the EPI program since 1989. Our group investigated the impact of HB vaccination regarding its efficacy as part of Thailand's EPI and found the prevalence of HBsAg distribution among children who were born after HB vaccine integration to be only 0.7% (Poovorawan *et al*, 2000b)

The protective efficacy of a primary course comprised of 3 doses of vaccine is well established. However, the duration of protection is still unknown, especially when anti-HBs titers decline to low or undetectable levels. The routine boosters to sustain protection generally are not necessary due to anamnestic antibody responses that follow exposure to HBV. In a follow-up of 465 hospital employees, around 30% who had an anti-HBs level below 10 mIU/ml up to 15 years after primary vaccination were found to be immunologically primed by a B-cell spot ELISA (Boland *et al*, 1995). Studies in children have demonstrated that vaccine-induced immunological memory persists for at least 12-15 years (West *et al*, 1994; Liao *et al*, 1999) and the duration of immunological memory lasted at least 10 years based on a study by Wainwright *et al* (1997).

Primed T cells also have a synergistic effect on the action of memory B cells. Cytokines released by primed T-helper cells can stimulate the development of cytotoxic T-cells and natural killer cells, which can directly attack virally infected cells. Several countries have a policy of administering booster doses only to certain risk groups, such as immunocompromised persons who may have clinically significant breakthrough infections.

After booster vaccination, anti-HBs titers rise significantly within 3-5 days (Jilg *et al*, 1989). The

Correspondence: Prof Yong Poovorawan, Viral Hepatitis Research Unit, Department of Pediatrics, Chulalongkorn University and Hospital, Rama IV Road, Bangkok 10330, Thailand.
Tel: (662) 256-4909; Fax: (662)256-4929
E-mail: Yong.P@chula.ac.th

rapid activation of memory B lymphocytes should prevent or reduce the severity of natural HBV infection which has an incubation period of 4-12 weeks. So far there have been no report of a vaccinated person who has had a transient, benign breakthrough infection which has gone on to develop into chronic liver disease.

In this paper we report on the response to a booster dose of recombinant HB vaccine in children with and without primary immunization in order to study the persistence of immunological B cell response.

MATERIAL AND METHODS

This study was extended from a previously reported study of a serological survey of the impact of the HB vaccine program in Thailand. The study protocol had been approved by the Ethics Committee, Ministry of Public Health, Thailand. The blood samples were taken during the course of October/November, 1999. Before acquiring the blood samples, the parents of all participants were informed as to the purpose of the study and their written informed consent was subsequently obtained. As for the coverage of HB vaccination, the parents were asked if their children had ever received the vaccine in the past and the data thus obtained were either confirmed by, or altogether retrieved from health records. The locations of sample collection comprised Lop Buri, Chon Buri, and Nakhon Si Thammarat, which are in the central, eastern and southern region of Thailand, respectively.

In total, 123 children were enrolled in the study. In order to be included into the study the subjects had to fulfil the following criteria: general good health, no chronic illness, not undergoing immunosuppressive therapy, no clinical signs or symptoms associated with either HIV infection or any immunodeficiency of different etiologies and negative HBsAg as well as anti-HBc. The subjects were divided into 4 groups according to the history of HB immunization and serum anti-HBs level. Group 1, 2, and 3 represented children without prior HB immunization, children who had received only 1 or 2 doses, and children who had received the complete series of 3 doses. Group 4 comprised children who had received 3 doses but had low serum anti-HBs (OD sample/ OD negative ratio <10). According to Thai EPI, hepatitis B vaccine is administered at birth, 2 and 6 months of age.

All subjects received a single dose of recom-

binant hepatitis B vaccine (10 µg/0.5 ml, SmithKline Biologicals, Belgium) following enrollment in the study. Injections were given intramuscularly in the upper arm (deltoid region). Blood samples were taken at 30 +/- 5 days after the injection. Sera were separated by centrifugation and kept at -20°C until tested. The specimens were subjected to enzyme-linked immunosorbent assay for detection of HBsAg (AUSZYME), anti-HBs (AUSAB) and anti-HBc (CORZYME), using commercially available ELISA kits (Abbott Laboratories, North Chicago, Ill) according to the manufacturer's specifications. The anti-HBs titer was expressed in mIU/ml by comparing with WHO Standard. Data were analyzed by determining the seropositive rates and geometric mean titers (GMT).

RESULTS

One hundred and twenty-three children, with ages ranging from 57 to 131 months, (mean ± SD = 85.9 ± 19.6) took part in this study. The sex, age distribution, seropositive rates and geometric mean titers (GMT) of anti-HBs after a booster dose for all 4 groups were shown in Table 1. Table 2 showed the GMT results for all 4 groups. All subjects in group 4 had GMT > 100 mIU/ml whereas seroconversion rates were 66.7, 83.3 and 94.5% in group 1, 2 and 3, respectively.

DISCUSSION

From our finding, it is interesting to discover that 66.7% of subjects who had never received HB vaccine seroconverted after a single dose of vaccine with high GMT (202; 95% CI = 88-467 mIU/ml). In our previous study of healthy adolescents, the seroconversion rate was 44.4% after the first HB vaccine dose with a GMT of 4 mIU/ml (Poovorawan *et al*, 1993). In a study of 343 healthy adults, none had anti-HBs level above 10 mIU/ml after the first dose of HB vaccine (Jilg *et al*, 1989). These results confirm that age is an important factor influencing the response to primary HB immunization. Jilg *et al* (1989) also demonstrated that persistence of specific antibodies depends on the peak anti-HBs level after basic immunization.

A study in adult subjects showed that persons with low titers after primary immunization had undetectable levels of anti-HBs 6 years after but had an anamnestic type of response to the booster dose (Chiaromonte *et al*, 1995). This phenomenon

Table 1
Immune response following a booster dose of recombinant hepatitis B vaccine.

Group	No.	Sex (Male/female)	Age (month)	GMT (95% CI;mIU/ml)	Percent sero positive
1. No prior vaccination	18	6/12	94.4 ± 20.9	202 (88-467)	66.7
2. Incomplete vaccination	6	1/5	114 ± 24.5	191 (94-389)	83.3
3. Complete vaccination	90	41/49	84.1 ± 17.7	166 (103-267)	94.5
4. Complete vaccination with low titer	9	3/6	71.7 ± 7.11	2,055 (772-5,469)	100

Table 2
Distribution of vaccinees on the basis of anti-HBs titers developed after the injection.

Group	Anti-HBs titer (mIU/ml)					
	>10,000 (%)	1,001-10,000 (%)	101-1,000 (%)	11-100 (%)	<10	Negative (%)
1	0	3/18 (16.7)	4/18 (21.1)	5/18 (27.8)	0	6/18 (33)
2	0	0	4/6 (66.7)	1/6 (16.7)	0	1/6 (16.7)
3	2/90 (2.2)	19/90 (21.1)	29/90 (32.2)	25/90 (27.8)	10/90 (10.9)	5/90 (5)
4	1/9 (7.1)	5/9 (71.4)	3/9 (21.4)	0	0	0

was also observed for subjects in groups 3 and 4. Subjects in group 3 may have had low titers after primary vaccination, explaining their undetectable titers at screening. Almost 95% of subjects in group 3 and 100% of subjects in group 4, all of whom had received complete primary immunization, were seroconverted after the booster dose. These subjects may have developed cell-mediated immunity without a humoral response during primary vaccination, with the humoral response only developing after booster vaccination. This study confirms that HB vaccine - induced immunological memory lasted at least 8 years in children who received primary HB immunization with undetectable anti-HBs. Liao *et al* (1999) found that protective efficacy against chronic HBsAg carriage was 96% at 15 years after vaccination. Coursaget *et al* (1994) reported a protective efficacy against HBsAg of 88% in children at 9-12 years after vaccination, with no difference in efficacy between groups with and without a booster dose. Our long term follow-up of vaccinated neonates showed that protective antibodies persisted for 10-12 years in > 95% of vaccinees, irrespective of a booster dose given at the age of 5 years (Poovorawan *et al*, 2000a).

The European Consensus Group on Hepatitis B Immunity does not recommend routine boosters

for healthcare workers, in whom adequate immunological priming has been achieved, even if anti-HBs titers decline after vaccination (Anonymous, 2000). On the other hand, Yoshida and Saito (2000) found that 56% of healthcare workers had anti-HBs titers below 100 mIU/ml at 28 months after the vaccination and that the titers did not rise significantly within 7 days after the booster vaccination (Yoshida and Sai, 2000). They suggested that persons at occupational risk of HBV infection with anti-HBs below 100 mIU/ml should have a booster to maintain seroprotection. The children in group 3 who were not seroconverted might be non-responders and require further injections. Chiaramonte *et al* (1995) reported about 25% of non-responders to primary HB immunization were seroconverted after the fourth dose. Reasons for lack of antibody response are not clear but may be multifactorial and genetically related (Hess *et al*, 1992; Nowicki *et al*, 1985; Weissman *et al*, 1988).

In conclusion, our results show that B cell memory can recognize the antigen. The booster vaccination during 5 to 10 years after complete vaccination is not necessary in healthy. Further studies are needed to elucidate the duration of immunological memory and thus when and for whom booster vaccinations should be recommended.

ACKNOWLEDGEMENTS

The authors would like to express their profound gratitude to Dr Kasemporn Chaiear and to the entire staff of the provincial hospitals participating in the project. We also would like to thank the National Research Council, the Thailand Research Fund, Senior Research Scholar for financial support, SmithKline Beecham, Thailand for providing hepatitis B vaccine and Mrs Pisane Saiklin for editing our manuscript.

REFERENCES

- Anonymous. Are booster immunisations needed for lifelong hepatitis B immunity? European Consensus Group on Hepatitis B Immunity. *Lancet* 2000; 355: 561-5.
- Boland GJ, de Gast GC, Italiander E, van der Reijden J, van Hattum L. Long-term immunity to hepatitis B infection after vaccination with recombinant hepatitis B vaccine [Abstract]. *Hepatology* 1995; 22: 325.
- Chiaromonte M, Ngatchu T, Majori S, *et al.* Response to an extra dose of hepatitis B vaccine and specific antibody persistence in non-responders to primary immunisation. *Scand J Gastroenterol* 1995; 30: 601-03.
- Coursaget P, Leboulleux D, Soumare M, *et al.* Twelve-year follow-up study of hepatitis B immunization of Senegalese infants. *J Hepatol* 1994; 21: 250-4.
- Hess G, Hingst V, Cseke J, Bock HL, Clemens R. Influence of vaccinations schedules and host factors on antibody response following hepatitis B vaccination. *Eur J Clin Microbiol Infect Dis* 1992; 11: 334-40.
- Jilg W, Schmidt M, Deinhardt F. Four-year experience with a recombinant hepatitis B vaccine. *Infection* 1989; 17: 70-6.
- Liao SS, Li RC, Li H, *et al.* Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. *Vaccine* 1999; 17: 2661-6.
- Nowicki MJ, Tong MJ, Bohman RE. Alterations in the immune response of nonresponders to the hepatitis B vaccine. *J Infect Dis* 1985; 152: 1245-8.
- Poovorawan Y, Pongpunlert W, Theamboonlers A, *et al.* Randomized, single-blind comparison of the immunogenicity and reactogenicity of 20 µg and 10 µg doses of hepatitis B vaccine in adolescents. *Southeast Asian J Trop Med Public Health* 1993; 24: 255-9.
- Poovorawan Y, Sanpavat S, Theamboonlers A, Safary A. Long term follow-up (11 to 13 years) of high-risk neonates vaccinated against hepatitis B. *Antiviral Therapy* 2000a;5 (suppl 1) : 24.
- Poovorawan Y, Theamboonlers A, Vimolket T, *et al.* Impact of hepatitis B immunization as part of Thailand's EPI. *Vaccine* 2000b; 19: 943-9.
- Treadwell TL, Keeffe EB, Lake J, *et al.* Immunogenicity of two recombinant hepatitis B vaccines in older individuals. *Am J Med* 1993; 95: 584-8.
- Wainwright RB, Bulkow LR, Parkinson AJ, Zanis C, McMahon BJ. Protection provided by hepatitis B vaccine in a Yupik Eskimo population: results of a 10-year study. *J Infect Dis* 1997; 175: 674-7.
- Weissman JY, Tsuchiyose MM, Tong MJ, Co R, Chin K, Ettenger RB. Lack of response to recombinant hepatitis B vaccine in nonresponders to the plasma vaccine. *JAMA* 1988; 260: 1734-8.
- West DJ, Watson B, Lichtman J, Hesley TM, Hedberg K. Persistence of immunologic memory for twelve years in children given hepatitis B vaccine in infancy. *Pediatr Infect Dis J* 1994; 13: 745-7.
- World Health Organization. WHO expanded programme on immunisation. Report of 14th Global Advisory Group (Antalya, Turkey, Oct 14-18, 1991). Geneva: WHO, WHO/EPI/GEN/92.1, 1991.
- Yoshida T, Saito I. Hepatitis B booster vaccination for healthcare workers. *Lancet* 2000; 355: 1464.