STUDIES ON HEPATITIS B VACCINATION IN NEONATES

STUDIES ON HEPATITIS B VACCINATION IN NEONATES

Onderzoek naar hepatitis B vaccinatie van pasgeborenen

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam,
op gezag van de rector magnificus
Prof. dr. P. W. C. Akkermans M. Lit.
en volgens besluit van het College van Dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 22 december 1993 om 15.15 uur

door

Riwka del Canho

geboren te Rotterdam

Acknowledgement

This study has generously been supported by the Netherlands Prevention Fund and performed at the Department of Internal Medicine II of the University Hospital Dijkzigt in Rotterdam. Financial support for this thesis was kindly given by Merck Sharp & Dohme Vaccine Division, producer of HB-VAX-DNA®.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Canho, R. del

Studies on hepatitis B vaccination in neonates / R. del Canho. -
[S.l. : s.n.]. - ill.: Erasmus Universiteit Rotterdam - Rotterdam, 1993 -
Thesis Rotterdam. - with ref. - with summary in Dutch.
ISBN 90-9006689-6
Subject headings: hepatitis B vaccination / neonates

Omslag: Aimée Bentinck
Drukwerk: Mebo, Amsterdam

Promotiecommissie

Promotor:	Prof. dr. S.W. Schalm
Overige leden:	Prof. J.H.P. Wilson
	Prof. dr. H.J. Neyens
	Prof. dr. G.C. de Gast

Voor de kinderen die hebben meegedaan
aan dit onderzoek

Contents

Abbreviations

ALAT	alanine amino transferase
ASAT	aspartate amino transferase
anti-HBc	antibodies against hepatitis B core antigen
anti-HBe	antibodies against hepatitis B e antigen
anti-HBs	antibodies against hepatitis B surface antigen
CI	confidence interval
DNA	deoxyribonucleic acid
DTTP	diphtheria-tetanus-pertussis-polio
EPI	expanded program on immunization
GMT	geometric mean titer
HBIg	hepatitis B immunoglobulin
HBV	hepatitis B virus
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HLA	human leucocyte antigen
IU/L	international units/liter
PCR	polymerase chain reaction
RIA	radioimmunoassay
WHO	world health organization

Chapter 1

Introduction

Introduction

Hepatitis B virus

In 1965 Blumberg et al. published their classic paper in which the discovery of australia antigen was described (1). In 1968 the australia antigen was identified as hepatitis B virus surface antigen, causing serum hepatitis (2,3). For his breakthrough in hepatitis B research, Blumberg was awarded in 1976 with the Nobel Prize in physiology and medicine.

The hepatitis B virus is a double-stranded DNA virus, belonging to the group of hepadna viridae (4,5).

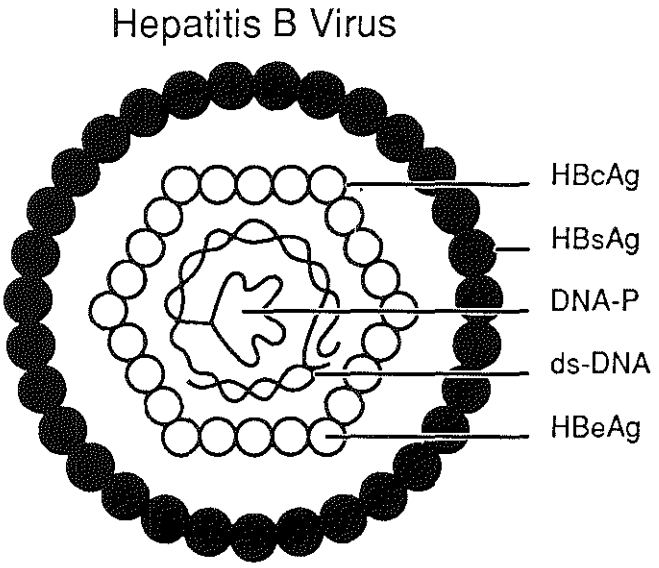

Figure 1. Model of the hepatitis B virus: DNA-P: DNA polymerase; ds-DNA: double-stranded DNA; HBsAg: hepatitis B surface antigen; HBcAg: hepatitis B core antigen; HBeAg: hepatitis B e-antigen.

Hepatitis B vaccine

Active immunization against hepatitis B was first introduced in 1970 by Krugman et al (6). A vaccine was prepared in which boiled diluted plasma from an Australia antigen carrier stimulated antibody in human subjects and protected against hepatitis B infection. This paved the way for manufacturing hepatitis B

vaccine. From pooled plasma of asymptomatic, healthy, high titre HBsAg carriers, HBsAg particles were derived for the preparation of plasma-derived hepatitis B vaccine. Inactivations steps insured non-infectivity and adjuvants were added as a preservative. The limited supply of suitable plasma of HBV carriers and the time- and money- consuming procedures involved to assure safety and purity led to the development of recombinant hepatitis B vaccines. The vaccine is prepared from hepatitis B surface antigen produced in recombinant baker's yeast (Saccharomyces cerevisiae) (7). Adverse reactions are minimal and consist of transient pain and swelling at the site of injection in 10-15% of vaccinees. Another 1-3% of recipients suffer from low-grade fever and malaise after administration of hepatitis B vaccine.

History of perinatal hepatitis B vaccination

Hepatitis B virus (HBV) infection and its sequelae, cirrhosis and hepatocellular carcinoma, has been a vaccine-preventable disease for a decade. Hepatitis B is still a major public health problem: over half of the world population has been infected with HBV, 350.000.000 people are chronically infected HBV carriers, who constitute the reservoir of infection. Forty percent of persisitently infected people will die of the consequences of the disease (8). The carrier rate of HBsAg varies world-wide from less than 1% in countries as Britain, Scandinavia, US and the Netherlands to more than 3% in Greece and Italy and up to 10-15% in Africa and the Far East (9). Perinatal infection of newborns with HBV from HBsAg carrier mothers is one of the main causes of HBV infections and of maintenance of the HBV reservoir (10-12). In 1982, a study was initiated in the Netherlands in which it was determined that in three test areas, pregnant women could be identified as HBsAg carriers by screening and that combined passive-active immunization of newborns of HBsAg carrier mothers could be carried out, leading to active development of sufficient anti-HBs in over 90% of cases (13,14). Results of 24-months follow up were described in the dissertation of Mazel: "Prevention of perinatal hepatitis B in neonates in the Netherlands" (15). These outcomes were in agreement with several other studies in the early 1980's, which showed that passive-active immunization of newborns of HBsAg positive mothers was safe, effective and immunogenic in 80-100% of cases (16-19). On the basis of the results of these studies, the health authorities in the Netherlands decided, in 1989, to introduce a national program to test all pregnant women for HBsAg and to administer passive-active hepatitis B immunization to newborns of HBsAg positive mothers (20). It was estimated that, with a mean birth rate of 170.000, this program could prevent 400 HBV infected neonates (probably leading to 15 acute hepatitis B, 1 fulminant hepatitis B, 250 HBV carriers) yearly in the Netherlands (15). Since the human hepatitis B virus has no known animal reservoir, a systemic vaccination

program against hepatitis B, including vaccination of all newborns and young children could theoretically lead to the eradication of hepatitis B. The WHO Expanded Program on Immunization has called for all countries with an HBV carrier prevalence of 8% or greater to have universal vaccination integrated into their routine immunization programs by 1995, and for all other countries by 1997 (21). Adapted guidelines are therefore needed in the Netherlands, with a estimated HBV carrier prevalence of 0.8% (14), for the introduction of national hepatitis B immunoprophylaxis for all newborns.

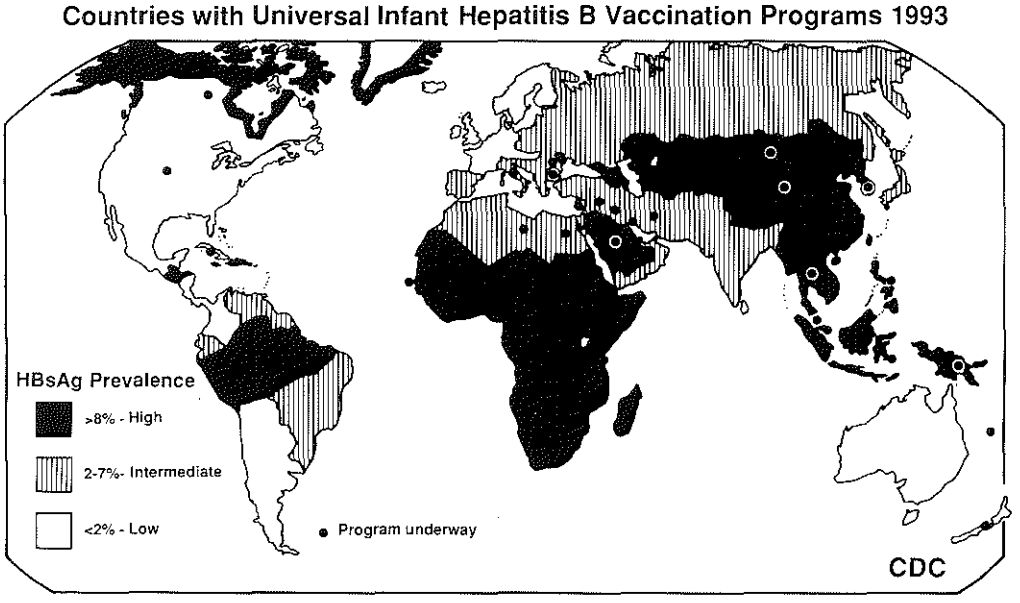

Figure 2. Hepatitis B vaccine policy of infant immunization in 1993. (from M. Kane, World Health Organization, Geneva, Switserland; with permission).

Failure of hepatitis B immunization in infants

Inadequate responses to hepatitis B vaccine occur in 2-4% of properly vaccinated infants of HBsAg-positive carrier mothers: 1-2% become HBsAg positive and another 1-2% are not able to develop sufficient amounts of anti-HBs for longlasting immunity against HBV. An anti-HBs antibody level of more than 10 IU/L is considered to provide protection against hepatitis B infection (22), anti-HBs of more than 100 IU/L for long-lasting immunity (23). Failure of neonatal immunoprophylaxis could be due to either in utero infection (17), a high dose of HBV, transmitted during delivery and related to maternal high-level viraemia

(24,25), insufficient neutralization capacity of the HBIg injection or failure to respond adequately to the vaccine, given in an adequate dose, due to genetic (26) or acquired causes (27-29). The question remained if preterm infants have adequate responses to hepatitis B immunization in comparison with full term infants.

Aims of the thesis:

1. To assess efficacy, immunogenicity and safety of different hepatitis B immunization schedules (dose, number of doses and time of onset vaccination) in neonates of HBsAg positive mothers (chapter 2 and 5) and in neonates of HBsAg negative mothers (chapter 3 and 4).

2. To assess hepatitis B vaccination in infants, with a gestational age of less than 37 weeks (chapter 6).

3. To investigate additional hepatitis B vaccination in non-infected neonates, with inadequate response after primary vaccination (chapter 7).

4. To evaluate causes of failure of neonatal hepatitis B vaccination (chapter 8 and 9).

5. To evaluate the follow-up of infants who became infected with hepatitis B in spite of passive-active hepatitis B immunization (chapter 10).

References

1. Blumberg BS, Alter HJ, Visnich S. A "new" antigen in leukamia sera. JAMA 1965; 191: 541-6.
2. Krugman S, Giles JP, Hammond J. Infectious hepatitis. Evidence for two distinctive clinical, epidemiological and immunological types of infection. JAMA 1976; 200: 365-73.
3. Prince AM. An antigen detected in the blood during the incubation phase of serum hepatitis. Proceedings National Academy of Sciences (Washington) 1968; 60: 814-21.
4. Summers J. Three recently described animal virus models for human hepatitis B virus. Hepatol 1981; 1: 179-83.
5. Tiollais P, Pourcel C, Dejean A. The hepatitis B virus. Nature 1985; 317: 489-95.
6. Krugman S, Giles JP, Hammond J. Viral hepatitis, type B (MS-2 strain): Studies on active immunization. JAMA 1971; 217: 41-45.
7. McAleer WJ, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. Nature 1984; 307: 178-80.
8. Ghendon Y. WHO strategy for the global elimination of new cases of hepatitis B. Vaccine 1990; 8: S129-33.
9. London WT, Blumberg BS. Comments on the role of epidemiology in the investigation of hepatitis B virus. Epidemiol Rev 1985; 7: 59.
10. Schweitzer IL. Vertical transmission of the hepatitis B surface antigen. Am J Med Sciences 1975; 270: 287-91.
11. Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975; 292: 771-4.
12. Ypma TjD. Studie over verticale transmissie van hepatitis B virus en de betekenis ervan voor de epidemiologie van hepatitis B-virus infecties. Proefschrift 1979. Utrecht.
13. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ et al. Passive-active immunisation of neonates of HBsAg positive carrier mothers: preliminary observations. Br Med J 1984; 288: 513-5.
14. Schalm SW, Mazel JA, de Gast GC, Heijtink RA, Botman MJ, Bänffer JRJ et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatr 1989; 83: 1041-7.
15. Mazel JA. Preventie van perinatale hepatitis B bij pasgeborenen in Nederland. Proefschrift 1986. Rotterdam.
16. Beasley RP, Hwang LY, Lee GC, Lan C, Roan CH, Huang FY et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-102.
17. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY et al. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984; i: 921-6.
18. Stevens CE, Toy PT, Tong MJ, Taylor PE, Vyas GN, Nair PV et al. Perinatal hepatitis B virus transmissionn in the United States: prevention by passive-active immunization. JAMA 1985; 253: 1740-5.
19. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'Doye R et al. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986; ii: 1143-5.
20. Geneeskundige Hoofdinspectie van de Volksgezondheid. GHI bulletin. Preventie hepatitis B bij pasgeborenen. September 1989.
21. World Health Organization. Expanded Programme on Immunization. Weekly Epidemiol Rec 1992; 3: 12-13.
22. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. Hepatology 1981; 1: 377-85.
23. Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986; 315: 209-14.

24. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989; i: 406-10.
25. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP et al. Prevention of maternal-infant hepatitis B virus transmission by immunization: The role of serum hepatitis B virus DNA. Hepatol 1986; 6: 369-73.
26. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321: 708-12.
27. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing hemodialysis. Acta Paediatr Scand 1985; 74: 213-8.
28. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H et al. Hepatitis B vaccination and immune respons in children with malignant diseases. Eur J Pediatr 1985; 144: 160-3.
29. Zuin G, Principi N, Tornaghi R, Paccagnini S, Re M, Massironi E et al. Impaired response to hepatitis B vaccine in HIV infected children. Vaccine 1992; 10: 857-60.

Chapter 2

Passive active immunization of infants of hepatitis B e antigen-positive mothers: comparison of the efficacy of early and delayed active immunization

Grosheide PM, Canho del R, Heijtink RA, Nuijten ASM, Zwijnenberg J, Bänffer JRJ, Wladimiroff YW, Botman MJ, Mazel JA, de Gast GC, Christiaens GCML, Gerards LJ, Fetter WPF, Baerts W and Schalm SW

American Journal of Diseases in Children: in press

Abstract

Objective: To assess the efficacy of late active immunization against hepatitis B concomitant with diphtheria, pertussis, tetanus and polio vaccine in high-risk infants receiving hepatitis B immune globulin at birth.

Design: Randomized study of infants born to mothers positive for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg).

Setting: Three large city hospitals and one rural area providing prenatal care and obstetric services.

Subjects: Eighty neonates of HBsAg- and HBeAg-positive carrier mothers received 0.5 ml/kg of body weight hepatitis B immune globulin within two hours of birth and hepatitis B vaccine (10 μg) at 0, 1, 2 and 11 months of age (group A) or at 3, 4, 5 and 11 months of age concomitant with diphtheria, pertussis, tetanus and polio immunization (group B). A second dose of hepatitis B immune globulin was given to infants on schedule B at 3 months.

Main outcome measures: Blood samples were collected at 0, 3, 6, 11, and 12 months of age and tested for antibodies to hepatitis B core antigen (anti-HBc) and HBsAg. Follow-up visits were scheduled annually up to 5 years of age.

Results: Eight infants were excluded from analysis. During the study period six children became HBsAg carriers, three in each group, which corresponds to a five-year incidence of infection of 9% and 8% for groups A (three of 35) and B (three of 37), respectively. Subclinical infections (persistent anti-HBc positivity beyond month 12 or appearance of anti-HBc) were encountered in another eight infants (four in each group).

Conclusion: Late active immunization starting at 3 months of age appears to provide similar protective efficacy as active immunization starting at birth when combined with hepatitis B immune globulin at 0 and 3 months of age.

Introduction

The current goal of immunoprophylaxis for newborns of mothers positive for hepatitis B surface antigen (HBsAg) is prevention of the chronic hepatitis B carrier state.

In June 1982 plasma-derived hepatitis B vaccine was licensed in the Netherlands and in July 1982 a program was initiated to determine a practical and effective immunization schedule for the prevention of hepatitis B in neonates of HBsAg-positive mothers. Because more than 95% of the relevant population participates in the national immunization program in the Netherlands, incorporation of hepatitis B vaccine into the diphtheria, pertussis, tetanus and polio (DPTP) vaccination program is presumed to yield the highest compliance and the lowest costs. Therefore we investigated two schedules of passive-active immunization to find out whether the simultaneous injection of hepatitis B vaccine and DPTP vaccine (delayed active immunization) provided the same protection against perinatal infections from HBsAg- and hepatitis B e antigen (HBeAg)-positive carrier mothers as early active immunization starting directly after birth.

Preliminary results of this study were reported earlier (1-2). Then, 34 infants of HBsAg- and HBeAg-positive mothers had entered the study. The final results on the protective efficacy of passive-active immunization in 80 high-risk infants after a follow-up of five years are reported herein.

Methods

Participating centers

The study was performed in four centers in the Netherlands: three large city hospitals in Utrecht (n=1) and Rotterdam (n=2) and the rural area Twente Gelderse Achterhoek where the number of home deliveries is high.

Ethics

Early in 1982 permission for the study was obtained from the local Medical Ethics Committees.

Hepatitis B screening

In July 1982 HBsAg screening of pregnant women was started in the four centers. Blood samples obtained from all pregnant women during their first visit to

the prenatal clinic of the participating centers were tested for the presence of HBsAg. Pregnant women with a positive test during the initial visit underwent a repeat test for HBsAg at week 28 of pregnancy. A woman was considered a HBsAg-positive carrier if the repeat test was positive for HBsAg. At the prenatal visit following the diagnosis of HBsAg carriership, the mother was informed about the immunization study program. Informed consent was obtained from the mother and information on country of birth and parity was noted in the woman's file (baseline characteristics). Randomization for either early or delayed active immunization took place at the local center.

At delivery an additional blood sample was obtained from the mother to verify the eligibility of her infant for the study. When a pregnant woman came to the two participating hospitals in Rotterdam for delivery and no HBsAg test result from prenatal visits was available, a rapid HBsAg test was performed. Results were available the next day before hospital discharge of the mother. If the rapid HBsAg test was positive, the mother was asked for informed consent and her baby was randomized.

All pregnant women who were positive for HBsAg were also tested for the presence of HBeAg. The enrollment of infants born to HBsAg- and HBeAg-positive mothers closed December 31, 1987.

Immunization schedules

Infants received an intramuscular injection of 0.5 ml/kg of body weight of hepatitis B immune globulin (HBIg) within 2 hours of birth except for infants of perinatally detected HBsAg carrier mothers who received HBIg as soon as possible. HBIg prepared by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, contained 100 to 150 international units of antibodies to the hepatitis B surface antigen (anti-HBs) per milliliter and was stored at 2°C to 8°C. The HBIg was given intramuscularly in the anterolateral region of the thigh by the individual assisting in the delivery.

For active immunization, infants were referred to a paediatrician. Infants on schedule A received 0.5 ml (10 μg) of plasma hepatitis B vaccine (HBvax) intramuscularly in the anterolateral thigh within two days of birth and 1, 2, and 11 months later, whereas those on schedule B received an equal dose of vaccine at 3, 4, 5, and 11 months of age. The vaccine (Merck Sharp & Dohme, West Point, Pa) contained 20 μg/ml of surface antigen and was stored at 2°C to 8°C. A second dose of HBIg (100 to 150 IU of anti-HBs/ml) was given at 3 months to infants receiving delayed active immunization according to schedule B.

Blood sampling

Immediately after delivery, cord blood was obtained from all infants. Follow-up blood samples were obtained at 3, 6, 11, and 12 months of age and then annually for at least five years.

Laboratory

Serum samples obtained from mothers during the study in Utrecht and Rotterdam (the Netherlands) were assayed for HBsAg by radioimmunoassay (Ausria-II, Abbott Laboratories, Chicago, Ill). The reversed passive hemagglutination assay (Auscell, Abbott) was used for initial screening in the Twente Gelderse Achterhoek area and for rapid HBsAg testing in Rotterdam. All positive samples detected by the reversed passive hemagglutination assay were confirmed by radioimmunoassay.When a mother was HBsAg-positive, the HBeAg status was assessed (Abbott-HBe). Serum samples from infants were assayed for anti-HBs by radioimmunoassay (Ausab, Abbott). The results were expressed in international units per liter (IU/l) with the aid of the World Health Organization reference serum. Samples obtained at birth and 12 months of age, as well as the annual samples, were also tested for antibodies to hepatitis B core antigen (Corab, Abbott). The presence of HBsAg was assayed in all samples with anti-HBs values below 100 IU/l.

Hepatitis B infections

Among infants two types of hepatitis B infection were registered after birth. A hepatitis B virus (HBV) infection was considered to have occurred if an infant became positive for HBsAg (cord blood excluded) or anti-HBc without HBsAg was observed after month 12 (3-4). Anti-HBc positivity had to be present on two or more consecutive occasions to rule out false low-level anti-HBc reactivity. Anti-HBc positivity without HBsAg at month 12 was considered to be due to maternal transmission of anti-HBc. Anti-HBc IgM was not considered to be a representative marker of acute hepatitis B infection since infants of HBsAg carrier mothers lacked this marker when infected perinatally (5). An increase in the anti-HBs level alone was not considered an HBV infection. An infant who was HBsAg-positive for more than six months was considered to be an HBsAg carrier (3). Infants who had no HBsAg-positive serum samples but were anti-HBc positive after month 12 were considered to have had a subclinical infection with HBsAg.

Randomization

From July 1, 1982 until December 31, 1987 all HBsAg-positive mothers admitted to the study were randomly assigned to one of two treatment groups. Randomization according to Peto et al occurred in each local center separately using numbered sealed opaque envelopes (6). Each block of six envelopes contained three cards with treatment A and three cards with treatment B. Stratification according to prenatal and perinatal detection of HBsAg was applied in the hospitals in Rotterdam since strict adherence to immunization schedules could not be guaranteed for the infants of perinatally detected HBsAg-positive mothers.

Statistics

The number of infants born to HBeAg-positive HBsAg carrier mothers available during the enrollment period determined the sample size.

The degree of similarity between the two treatment groups was demonstrated by comparing baseline characteristics of the HBsAg- and HBeAg-positive mothers. The baseline characteristics were compared for all mothers who agreed to treatment of their infants (n=80) and for mothers whose infants received proper treatment according to schedule A or B (n=72). Differences in proportions related to country of birth of the mothers, parity, and rapid screening between treatment groups were compared using the Chi-square test and Fisher's exact test in the case of small numbers. Median ages of mothers were compared using the Wilcoxon test. Differences in number of HBV events between groups were calculated with the Fisher's exact test and 95% confidence intervals (CI). The exact values for 95% CI are given in Geigy Scientific Tables (7).

Results

A total number of 527 pregnant women was found to be repeatedly HBsAg-positive by screening; 92 (17%) of these mothers were also HBeAg-positive. Eighty infants of HBsAg- and HBeAg-positive mothers were born in the study period and eligible for passive-active immunization (38 according to schedule A; 42 according to schedule B).

Eight infants (schedule A, three infants; schedule B, five infants) were excluded from further analysis: three infants never received vaccine (schedule A, one infant; schedule B, two infants), three infants received two doses of vaccine but no serum sample was available after month 0 (schedule A, two infants;

Table 1. Baseline characteristics of HBsAg- and HBeAg-positive mothers of infants immunized according to schedule A or B.

Mothers	Schedule A n = 35	Schedule B n = 37	P-value
Median age, years	23 (19;33)*	24 (17;38)	P=0.06†
Country of birth, No (%)			P=0.92°
Netherlands + other	3 (8.6%)	2 (5.4%)	
Mediterranean	15 (42.9%)	16 (43.2%)	
Surinam	5 (14.3%)	7 (18.9%)	
Asia	12 (34.3%)	12 (32.4%)	
Rapid screening, No (%)	2 (5.7%)	2 (5.4%)	P=1.00§
Primigravidae, No (%)	16 (45.7%)	10 (27.0)	P=0.10°

* Numbers in parentheses indicate fifth to 95th percentile.
† Wilcoxon test.
° Chi-square test.
§ Fisher's exact test.

Table 2. Time at which infants who became HBsAg carriers despite passive-active immunization were first positive for HBsAg.

		HBsAg-positive						anti-HBs in IU/1				
case	schedule	0†	3	6	11	12	months	0†	3	6	11	12
1	A	+	-	+	+	+		0	16	2	0	0
2	A	+	-	-	+	+		0	45	39	0	0
3	A	+	+	+	+	+		0	0	0	0	0
4	B	+	+	+	+	+		0	0	0	0	0
5	B	+	-	+	+	+		0	29	0	0	6
6	B	-	-	-	+	+		0	38	17	0	0

Plus sign (+) indicates HBsAg-positive and minus sign (-) indicates HBsAg-negative.
† Cord blood.

schedule B, one infant), and one infant died of congenital abnormalities 10 days after birth (schedule B, one infant). One infant received the wrong immunization schedule (schedule B, one infant), but the last available blood sample tested was negative for HBsAg.

In Table 1 baseline characteristics are presented for the 72 mothers whose infants received proper treatment. The baseline characteristics of these 72 mothers did not differ from those for the total group of 80 mothers originally randomized.

HBsAg positivity was found in three (9%) of 35 infants in group A (CI, 1.8 to 23.1) and three (8%) of 37 infants in group B (CI, 1.7 to 21.9). All HBsAg-positive infants became carriers. The onset of HBsAg positivity in those infants who could not be protected occurred within the first 11 months of life (Table 2). Two infants were already HBsAg-positive in cord blood and thereafter. The other four carrier infants were first HBsAg-positive at 6 or 11 months of age; their anti-HBs antibody levels varied from 16 IU/l to 45 IU/l at month 3 and were absent from month 6 onwards in all but one case. Infant 5 had concurrent HBsAg and anti-HBs at month 12.

Four (13%) of 32 infants in group A (CI, 3.5 to 29.0) and four (12%) of 34 infants in group B (CI, 3.3 to 27.5) exhibited anti-HBc beyond month 12 on at least two consecutive occasions; in all cases the development of anti-HBs was normal during the first 12 months of age (Table 3). Four infants (patients 1 through 4), two in each group, were anti-HBc positive from month 12 onwards. The other four infants became anti-HBc positive after month 12. Anti-HBs titers varied considerably in these anti-HBc-positive infants and either increased or (slowly) decreased. HBsAg was never observed in these infants.

The frequency of HBV events per group, seven (20%) of 35 infants in group A (CI, 8.4 to 36.9) and seven (19%) of 37 infants in group B (CI, 8.0 to 35.2), was not statistically different. The 95% CI for the difference between the two population proportions ranges from -17% to 19%.

The HBsAg- and HBeAg-positive mothers who infected their infants were all detected during pregnancy; they had a normal pregnancy and spontaneous delivery. The six infants who became HBsAg carriers received HBIg directly after birth and, except for patient 5 who received the second vaccination 3 weeks late, all infants were immunized according to schedule.

To determine whether mothers from Asia with suspected high levels of the virus (8,9) were more likely to infect their offspring, we evaluated the ethnic distribution of the HBsAg- and HBeAg-positive carrier mothers in relation to the perinatal transmission of HBV. The ethnic distribution of mothers with HBV infected infants was similar in groups A and B (Table 4). When HBV infected infants from the two groups were combined and analyzed according to maternal origin, differences became apparent. In contrast to our expectations, the rate of

Table 3. Appearance/persistence of anti-HBc and anti-HBs in follow-up sera from infants with subclinical infection despite passive-active immunization according to schedule A and B.

case	sche-dule	anti-HBc at month 12	24	36	48	60	anti-HBs in IU/L at month 12	24	36	48	60
1	A	+	+	+	+	NT	1299	2371	7643	4080	NT
2	A	+	+	NT	NT	+	1183	134	90	NT	34
3	B	+	+	+	+	+	559	3903	2962	1185	1037
4	B	+	+	+	+	+	25660	21555	10195	10989	NT
5	A	-	-	+	+	+	2280	332	309	301	144
6	A	-	+	+	+	+	4989	349	209	175	182
7	B	-	-	NT	+	+	2191	725	NT	1043	795
8	B	-	-	+	+	NT	898	78	110	32	NT

NT indicates no test result available
Plus sign (+) indicates anti-HBc positive
Minus sign (-) indicates anti-HBc negative.

Table 4. HBV infections (HBsAg carriers and anti-HBc positivity after month 12) by study group and ethnic distribution.

Country of birth	Number of infants: Schedule A	B	Total	Number of HBV infections: Schedule A	B	Total
Mediterranean	15	16	31	5 (2)	6 (3)	11 (5)
Asia	12	12	24	2 (1)	1 (-)	3 (1)
Total	27	28	55	7 (3)	7 (3)	14 (6)

Number in parentheses indicate number of HBsAg carrier infants

transmission of HBV for mothers of Asian origin (three [13%] of 24) tended to be lower (CI, 2.7 to 32.4) than that for mothers of Mediterranean origin (11 [36%] of 31; CI, 19.2 to 54.6). This trend in frequency of HBV infections was just above statistical significance (P=0.05; Chi-square test).

Discussion

This study demonstrates that the frequency of hepatitis B antigenemia for infants of HBsAg- and HBeAg-positive mothers after delayed active immunization, starting at 3 months, is similar to that obtained with early active immunization starting directly after birth: 8% and 9%, respectively. Only six (8%) of 72 infants (CI, 3.1 to 17.3) became HBsAg carriers, a rate comparable with that found in other passive-active immunization studies with either plasma vaccine or recombinant vaccine (8,10-14). Our results are in agreement with the findings of Beasley et al. who demonstrated HBsAg carrier rates of 2% for the delayed vaccination also starting at 3 months vs 6% for the early vaccination given 4 to 7 days after birth (10).

Since the number of HBsAg-positive infants in both studies was relatively small and anti-HBc is the most sensitive marker of HBV infection, we also compared the number of anti-HBc positive infants without HBsAg in the two groups. Anti-HBc positivity was found for eight infants, four in each group. All subclinical infections were observed in infants with an active immune response to vaccine; none of these infants had development of the carrier state or became HBsAg-positive. This indicates that infants with an adequate initial anti-HBs response remained protected against HBV carrier state for at least five years. After the follow-up period of five years, the total number of hepatitis B infections was similar for both groups; seven (20%) in group A and seven (19%) in group B. The 95% CI for the difference between the two groups ranges from -17% to 19%, showing the imprecision due to the limited sample size. Further studies with larger numbers of neonates will be needed to confirm these results, although the low number of HBV infections suggests no major clinical relevance.

It is unlikely that the HBV infections were generated by vaccine-induced escape mutants (seen in Mediterranean countries) since these mutants are characterized by HBsAg and concomitant adequate levels of nonneutralizing anti-HBs (15).

In the Netherlands, where prenatal screening of pregnant women is current policy, immunoprophylaxis with vaccine alone is not considered and HBIg is always given at birth. The cardinal question then becomes what vaccination schedule is feasible with high compliance and what schedule is most effective? We tested one schedule we thought would be the most effective (0, 1, 2 and 11 months)

and one schedule we thought would be associated with highest compliance (3, 4, 5, and 11 months). The results showed no difference in efficacy, confirming the original observations by Beasley et al. (10), and higher immunogenicity (2). In this setting, the compliance with both schedules was similar but early active immunization does not correspond to the routinely scheduled immunization visits in practice.

In many countries, hepatitis B immunization starting immediately after birth is advocated. Although this approach is scientifically well founded, both the compliance related to multiple injections and the costs related to additional physician visits might interfere with high compliance to the vaccination programs. In our opinion, both our study and the study of Beasley et al. demonstrate that the timing of the start of active immunization within the period of 0 to 3 months after birth is of minor importance for neonates receiving HBIg directly after birth (10). This conclusion is of considerable importance when designing strategies for HBV immunization programs with a high degree of compliance. Current experience suggests that high compliance is difficult to achieve with many injections on separate months (16-17). In Italy, where mass vaccination against hepatitis B was recently introduced, the highest compliance rate was observed in the babies who received hepatitis B vaccination at the same time as the mandatory childhood vaccinations (98%), whereas it was 80% in babies who received the vaccines separately (16).

An extra dose of vaccine at month 0 with no proven benefit would mean additional effort and cost. Conversely, data on the immunogenicity and efficacy of a vaccination schedule starting at birth with a second dose at month 3 are unavailable. Increasing the interval between the first and the second dose of vaccine beyond the recommended interval of four weeks has been reported to decrease the antibody response (18).

If the hepatitis B vaccine could be given at the same time as DPT or DPT-polio immunization, the number of physician visits could be reduced; in addition, the number of injections could be reduced if hepatitis B vaccine could be incorporated into the DPT vaccine.

Furthermore, the influence of the vaccination schedule is demonstrated by significantly higher anti-HBs response in infants receiving late active immunization (1-2). Age at the first injection of vaccine is a matter of controversy because, on the one hand it is important to protect the neonates as early as possible, delivery being the most crucial time to get HBV infection; on the other hand, the physiological immunosuppression of newborns may produce a less effective response to vaccination.

A potential drawback of delayed active immunization may be the need for an additional HBIg injection, as was given to infants in our study as well as in the study

of Beasley et al. (10). The need for a second dose of HBIg with delayed vaccination, however, has not been established. In fact, an efficacy study showed no evidence of enhanced protection when an additional dose of HBIg is used (Grosheide et al.: submitted for publication). It seems reasonable to expect that active immunity will develop rapidly enough to eliminate the need for subsequent doses of HBIg (19).

In the Netherlands, a low prevalence area with a high percentage of home deliveries, vaccination starting directly after birth does not fit easily into the child care program; DPT-polio injections are given at the ages of 3, 4, 5 and 11 months. Passive immunization of infants of HBsAg-positive mothers immediately after birth by the midwife or obstetrician, followed by active immunization by the physician responsible for routine infant immunization, appears feasible. Because compliance to DPT-polio vaccination in the Netherlands reaches 95%, simultaneous administration of the hepatitis B vaccine with DPT-polio vaccination is likely to be associated with a similar high compliance for hepatitis B. Evaluation of infant immunization would occur within the existing health care system. At virtually no cost and without extra effort, infants can be immunized against hepatitis B. The results of this study open the way for general application of hepatitis B vaccine, incorporated into a DPT vaccine, in countries that provide hepatitis B immune globulin prophylaxis at birth to high-risk neonates.

Acknowledgement

We thank Marja Bakker-Bendik, Thea van der Laar-Kokx, Harriet Ribbers-Smeenk, Hetty C. van Dijk, Anneke Deurloo-Vollaard and Marja Voogd-Schotanus who made this study their fulltime professional effort for several years. Special thanks go to Jan Boot, Wim C.J. Hop, statistician and J. Ria Klokman-Houweling for their technical assistance.

This study was supported by a grant from the Praeventiefonds, the Hague, the Netherlands.

References

1. Mazel JA, Schalm SW, Gast de GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg-positive carrier mothers: Preliminary observations. Br Med J 1984;288:513-515.
2. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
3. Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sun TS, Hsieh FJ, Szmuness W. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Lancet 1981;2:388-393.
4. Szmuness W, Stevens CE, Harley EJ, Zang EA, Alter HJ, Taylor PE, DeVera A, Chen GTS, Kellner A, the dialysis vaccine trial study group. Hepatitis B vaccine in medical staff of hemodialysis units. Efficacy and subtype cross-protection. N Engl J Med 1982;307:1481-1486.
5. Goudeau A, Yvonnet B, Lesage G, Barin F, Denis F, Coursaget P, Chiron JP, Diop Mar I. Lack of anti-HBc IgM in neonates with HBsAg carrier mothers argues against transplacental transmission of hepatitis B virus infection. Lancet 1983;2:1103-1104.
6. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 1976;34:585-612.
7. Lenter C, (ed). Geigy Scientific Tables. 8th ed. Basel, Switzerland: Geigy;1982:89-102.
8. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier status in newborn infants in mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immune globulin: Double-blind randomized placebo-controlled study. Lancet 1984;1:921-926.
9. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W. Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. Hepatology 1983;3:135-141.
10. Beasley RP, Hwang LY, Lee GCY, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
11. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV, Weissman JY, Krugman S. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987;257:2612-2616.
12. Poovorawan Y, Sanpavat S, Pongpuniert W, Chumdermpadetsuk S, Sentrakul P, Safary A. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen-positive mothers. JAMA 1989;261:3278-3281.
13. Lee CY, Huang LM, Chang MH, Hsu EY, Wu SY, Sung JL, Safary A. The protective efficacy of recombinant hepatitis B vaccine in newborn infants of hepatitis B e antigen-positive-hepatitis B surface antigen carrier mothers. Pediatr Infect Dis J 1991;10:299-303.
14. Ip HMH, Lelie PN, Wong VCW, Mimms L, Reesink HW. Prevention of the HBV-carrier state in infants of HBsAg- and HBeAg-positive mothers with high and low serum levels of HBV-DNA. A 3 year placebo-controlled study comparing the efficacy of hepatitis B vaccine with and without hepatitis B immunoglobulin. Lancet 1989;1:406-410.
15. Carman WF, Zanetti AR, Karayannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutants of hepatitis B virus. Lancet 1990;336:325-329.
16. Da Villa G, Piazza RI, Picciotto L, Peluso P, Luca de G, Basile B. A pilot model of vaccination against hepatitis B virus suitable for mass vaccination campaigns in hyperendemic areas. J Med Vir 1992;36:274-278.
17. Stroffolini T, Pasquini P and collaborating group. Five years of vaccination campaign against

hepatitis B in Italy in infants of hepatitis B surface antigen carrier mothers. Ital J Gastroenterol 1990;22:195-197.

18. Inskip HM, Hall AJ, Chotard J, Loik F, Whittle H. Hepatitis B vaccine in the Gambian expanded programme on immunization: factors influencing antibody response. Int J Epidemiol 1991;20:764-769.

19. Prevention of perinatally transmitted hepatitis B infection. Lancet 1984;1:939-941. Editorial.

Chapter 3

Immunogenicity of a full dose (20 μg) of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes

Canho del R, Grosheide PM, Voogd-Schotanus M, Huisman WM, Heijtink RA, Schalm SW

Journal of Medical Virology 1993; 41: 30-34

Abstract

The immunogenicity of a full dose of recombinant DNA yeast-derived hepatitis B vaccine (Engerix-B) was assessed in healthy neonates in order to compare three candidate vaccination schemes. After randomization 162 newborns of HBsAg-negative mothers entered the study. Neonates received hepatitis B vaccine according to a four-dose vaccination scheme starting either at month 3 (scheme I; months 3, 4, 5 and 11) or at birth (scheme III; months 0, 1, 2 and 11). Another group of neonates received hepatitis B vaccine according to a three-dose scheme starting at birth (scheme II; months 0, 1 and 6). No serious adverse reactions were observed; 2.5% of the vaccinated newborns suffered mild transient local symptoms. The vaccine was highly immunogenic irrespective of vaccination scheme; all infants developed anti-HBs levels ≥ 10 IU/L, 97% ≥ 100 IU/L. The immunogenicity of hepatitis B vaccine after primary- and booster- vaccinations, administered in the four-dose scheme started at birth was significantly ($p < 0.05$) higher than in the three-dose scheme started at birth. Hepatitis B vaccination according to the four-dose scheme started at month 3 produced significantly ($p < 0.05$) higher antibody levels in comparison to the four-dose scheme started directly after birth. This study showed that a four-dose hepatitis B vaccination scheme starting at month 3 resulted in the highest antibody levels of the three schemes investigated and can be recommended for incorporation into the Expanded Programme on Immunization in the Netherlands.

Key words: hepatitis B vaccine, immunogenicity, neonates

Introduction

The WHO strategy for the control of hepatitis B virus (HBV) infection and its sequelae, chronic hepatitis, cirrhosis and hepatocellular carcinoma, is mass vaccination of infants and young children within the framework of the WHO Expanded Programme on Immunization (EPI) (Ghendon 1990). Plasma-derived hepatitis B vaccine (PDV) has proven to be safe, effective and immunogenic in newborns for almost ten years (Beasley et al., 1983; Coursaget et al., 1986). Since the introduction of hepatitis B recombinant DNA vaccine, the vaccine became widely available. Clinical trials with the recombinant DNA yeast-derived hepatitis B vaccine (YDV), Engerix-B, manufactured by SmithKline Beecham Biologicals were started in 1984 to determine its safety, efficacy and immunogenicity in humans. Initial trials carried out with young adults indicated that YDV is safe and immunogenic in man and that it is comparable to PDV in terms of safety and immunogenicity (Scheiermann et al., 1987; Goudeau et al., 1987). Later on clinical trials involving three groups with a high risk for hepatitis B infection, i.e. the institutionalized mentally handicapped, homosexual men and neonates of hepatitis B surface antigen and e antigen (HBsAg, HBeAg) positive mothers, showed that YDV is safe, highly immunogenic and effective since the rate of hepatitis B infection was reduced to less than 5% (Damme Van et al., 1989; Goilav et al., 1990; Poovorawan et al., 1990). Engerix-B was licensed in the Netherlands in 1987. The established dose of Engerix -B vaccine used for neonates of HBsAg-positive mothers in the Netherlands is 20 μg and the vaccine is given according a four-dose scheme at 3, 4, 5 and 11 months, together with the diphtheria-tetanus-pertussis-polio (DTPP) vaccine (Anonymous, 1989). In other countries, however, the recommended three-dose scheme is started soon after birth, using only half the dose: 10 μg (Centers for Disease Control, 1990). Until now, no studies on administration of the adult (20 μg) dose of Engerix-B to neonates of HBsAg-negative mothers have been published. Therefore we compared the immunogenicity of 20 μg Engerix-B YDV administered in two four-dose vaccination schemes starting either at month 3 or at birth. We also compared a four-dose scheme with a three-dose scheme, both starting at birth.

Methods

Subjects and randomization

All pregnant women attending the prenatal clinic of the University Hospital Dijkzigt Rotterdam, the Netherlands, were screened for the presence of HBsAg at

their first visit, together with the lues and blood group serology. HBsAg-negative mothers were informed about the current hepatitis B immunization programme. Informed consent was obtained from the mother for the participation of her infant. Country of birth and age of the mother, gestational age, birthweight and sex of the child were registered on the parturition form. To be accepted in the study, newborns had to weigh at least 2000 grs at birth and have a 5-minutes Apgar score of 7 or higher. 180 newborns were randomised into three groups (groups 1-3), according to Peto (Peto et al., 1976). 18 children did not start the study for the following reasons: secondary refusal of the parents (n=13), delivery outside the trial area (n=3), birthweight less than 2000 grs (n=1) and multiple congenital malformations (n=1). 51 neonates of group 1, 56 of group 2 and 55 of group 3 entered the study (intention to treat).

Vaccine and vaccination-schemes

The recombinant DNA yeast-derived hepatitis B vaccine was provided by SmithKline Beecham Biologicals (Engerix-B, Rixensart, Belgium). The vaccine was ad and ay antigenic and 20 μg HBsAg were contained in 1.0 ml in single dose vials (lot numbers 163A4, 189A4, 199A4). The production, quality control and physiochemical characteristics of Engerix-B have been described elsewhere (Wilde De et al., 1985; Peetermans et al., 1987). Vaccine was always stored at 2 - 8 °C and administered into the quadriceps muscle by a physician. Parents were asked to record any local (soreness, redness, swelling) or systemic (fever >37.5 °C, gastrointestinal symptoms, fatigue) symptoms during three days following each injection of vaccine. 51 neonates of group 1 received four doses of vaccine at months 3, 4, 5 (primary) and 11 (booster) -scheme I-; 56 neonates of group 2 received three doses of vaccine at months 0, 1 (primary) and 6 (booster) -scheme II- and 55 neonates in group 3 received four doses of vaccine at months 0, 1, 2 (primary) and 11 (booster) -scheme III-.

Blood tests and laboratory methods

2 ml of blood were obtained at month 0 (umbilical cord blood) and months 4, 6, 11 and 12 in group 1; at months 3, 6, 7 and 12 in group 2 and at months 3, 6, 11 and 12 in group 3.

HBsAg, anti-HBc and anti-HBs were measured by radioimmunoassay (Ausria II, Corab, Ausab, Abbott Laboratories Chicago USA). Anti-HBs is expressed in International Units/Litre (IU/L) after comparison with the WHO standard preparation (Central Laboratory of the Netherlands Red Cross Blood Transfusion Services, Amsterdam, the Netherlands).

Table 1. Distribution of the entry-variables by vaccination in groups 1, 2 and 3.

Scheme	I	II	III	P value
Number of infants (N)	51	56	55	
Median gestational age (weeks)	40	40	40	0.92*
Median birthweight (grams)	3330	3340	3240	0.40*
Sex of child: (N)				0.16†
Males	33	34	26	
Females	18	22	29	
Anti-HBs positive (N)	14	10	8	0.23†
Anti-HBc positive (N)	10	10	7	0.61†
Median age mother (years)	29	28	30	0.07*
Country of birth mother: (N)				0.36†
Netherlands	22	24	28	
Mediterranean	9	7	4	
Surinam	12	14	12	
Asian	0	2	5	
Miscellaneous	8	9	6	

* Kruskal Wallis test
† Chi-square test

Statistics

Statistical analysis was performed on two data sets: data according to intention-to-treat data and clean data (data of all children who entered the study and were vaccinated and assessed according to the protocol). Differences in discrete variables were analysed by the Chi-square test. Continuous variables were analysed by the Kruskal Wallis test and the two-sample Wilcoxon rank sum test for unpaired observations. Geometric mean anti-HBs levels (GMT) are expressed in IU/L with 95% confidence intervals (CI).

Results

From September 1988 to March 1990, 162 infants born of HBsAg-negative mothers entered the study. After primary vaccination serum samples were available

in 45 (88%), 54 (96%) and 53 (96%) cases in scheme I, II and III, respectively. After boostervaccination, these figures were: 42 (82%), 51 (91%) and 48 (87%). 7 children discontinued the study, 1 child due to bad compliance with the vaccination scheme and 6 parents refused further cooperation. Clean data are based on 155 children. Statistical comparison of clean data with intention-to-treat data did not reveal significant differences. The results of analysis of the clean data are presented below. There were no significant differences in country of birth of the mother, median age of the mother, median gestational age, median birthweight, sex of the child and number of infants with anti-HBs positivity and anti-HBc positivity in cord blood between the three groups (table 1).

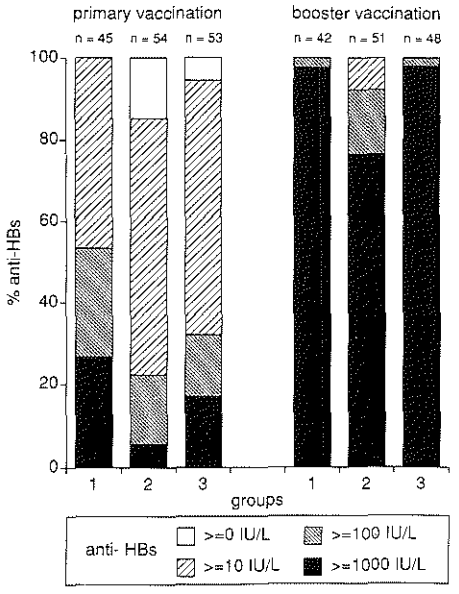

Figure 1. Percentage of infants with various anti-HBs levels after primary- and booster-vaccination with 20 μg Engerix-B hepatitis B vaccine according to scheme I: 3, 4, 5, 11 months; scheme II: 0, 1, 6 months and scheme III: 0, 1, 2, 11 months.

Passive transfer of maternal antibodies

Passively acquired anti-HBs positivity rates in cord blood were 27% (n=14), 18% (n=10) and 15% (n=8) for group 1, 2 and 3, respectively. Anti-HBc positivity rates at birth were 20% (n=10), 18% (n=10) and 13% (n=7), respectively. In all cases anti-HBc became negative at month 12.

Anti-HBs seroconversion rate

Figure 1 shows the frequences of protective levels of anti-HBs levels after primary- and booster-vaccination in the three different vaccination schemes. An anti-HBs level ≥ 10 IU/L is assumed to be protective against hepatitis B. All infants who received the first vaccination at month 3 (scheme I), developed anti-HBs levels ≥ 10 IU/L after primary vaccination. All infants who started vaccination at birth (scheme II and III), developed anti-HBs levels ≥ 10 IU/L, but in 11 cases this did not occur until after the booster vaccination. As far as long-term protection is concerned, all infants from group 1 and 3 had anti-HBs levels ≥ 100 IU/L after the booster vaccination. Whereas in group 2, this corresponding percentage

amounts only 92 (47/51). 98% of the infants in group 1 and 3 had anti-HBs titre ≥ 1000 IU/L after the booster vaccination in comparison with 76% of the infants in group 2.

The anti-HBs levels for 8 children in scheme II (15%) and 3 children in scheme III (6%) were < 10 IU/L after primary vaccination (month 3). After the booster vaccination 6 of these children exhibited more than 1000 IU/L, 3 between 100 and 1000 IU/L and 2 between 10 and 100 IU/L.

Table 2 lists the anti-HBs levels (GMT) with 95% CI (anti-HBs > 0 IU/L) for the three different schemes. The GMT anti-HBs after primary vaccination and after booster vaccination with 4 doses for scheme I, which started at month 3, were 264 IU/L and 20768 IU/L, respectively. This is significantly (Wilcoxon test: P value 0.004 and 0.01, respectively) higher than those for scheme III, which started at birth, i.e., 135 IU/L and 10495 IU/L, respectively. Comparison of the groups receiving 3 and 4 doses, both started at birth, shows that the GMT anti-HBs after primary- and booster-vaccination in four-doses (scheme III) were significantly (Wilcoxon test: P value 0.004 both) higher than those after 3 doses (scheme II): 56 IU/L and 3090 IU/L, respectively.

Table 2. Immunogenicity after primary- and booster- vaccination with 20 μg Engerix-B hepatitis B vaccine according to three schemes varying in number of doses and time of immunization.

Scheme	Month	anti-HBs (>0 IU/L) (GMT)	95 % CI	P value
1-2 months after primary vaccination				
I (3, 4, 5, 11)	6	264	157-444	
II (0, 1, 6)	3	56	44-106	0.004*
III (0, 1, 2, 11)	3	135	87-221	0.004†
1 month after booster vaccination				
I (3, 4, 5, 11)	12	20768	14714-29312	
II (0, 1, 6)	7	3090	1830-5218	0.004*
III (0, 1, 2, 11)	12	10495	7332-15023	0.010†

* Wilcoxon test scheme I versus II
† Wilcoxon test scheme I vesus III

No significant differences in GMT anti-HBs were found between boys and girls, presence of passively acquired maternal antibodies (anti-HBs, anti-HBc) and various countries of birth of the mother. Of the 32 infants with passively acquired anti-HBs at birth (GMT anti-HBs at month 0: 333 IU/L, 95% CI: 140-798 IU/L), 31 exhibited at least one increase in the anti-HBs titre after vaccination, only one did not.

Adverse reactions to vaccine

No cases of clinical hepatitis or serious side-effects of the vaccine were reported. In four cases (2.5%) the parents of the vaccinated children reported transient soreness at the site of injection. No fever was noted in groups 2 and 3 (after the first three doses). Most children in group 1 received DTPP vaccination synchronous with the hepatitis B vaccination. Due to the relatively frequent occurrence of fever, a side-effect known of DTPP vaccination, we were not able to evaluate the fever observed in group 1.

Discussion

The 20 μg Engerix-B hepatitis B vaccine used in this study was safe and highly immunogenic in all 3 vaccination schemes. No serious side-effects were reported. In only 2.5% of the infants mild transient local symptoms were present; this finding is in agreement with earlier results (Poovorawan et al., 1990). All infants reached anti-HBs levels ≥ 10 IU/L, 97% ≥ 100 IU/L, after completion of the vaccination scheme (figure 1). An anti-HBs level ≥ 10 IU/L is assumed to be protective against HBV infection (Szmuness et al., 1981) and an anti-HBs level ≥ 100 IU/L is taken as evidence of long-lasting immunity (Hadler et al., 1986). The anti-HBs level (GMT) at month 6 in our study is comparable to the concentration anti-HBs at month 6 in an earlier study, in which 20 μg Engerix-B YDV was administered to neonates of HBsAg-positive mothers in 3 doses at months 0, 1 and 2, without hepatitis B immunoglobuline (HBIg) administration (Cadranel et al. 1987). The immunogenicity demonstrated in our study was 3 times higher than that reported in a corresponding study in which only half of the dose (10 μg) of Engerix-B YDV was used (Poovorawan et al., 1990); it was similar to the acquired anti-HBs concentration reported for healthy adults receiving the full 20 μg dose of Engerix-B YDV (Scheiermann et al., 1987). The recommended dosage of vaccine for neonates is ambiguous; the Dutch authorities advises a full dosage of 20 μg Engerix-B YDV (Anonymous, 1989), the Immunization Advisory Committee recommends half of this standard adult dosage (10 μg) (Centers for Disease Control, 1990). The

duration of immunity after hepatitis B vaccination is currently unknown but it is generally assumed that the higher the antibody levels after vaccination, the longer the period of protection (Hadler et al., 1986; Jilg et al., 1987; Scheiermann et al., 1990). The vaccine was highly immunogenic in all 3 schemes investigated. For reasons of compliance it seems favourable for health authorities to opt for that scheme which can be incorporated into the existing EPI programme. In this study, Engerix-B YDV administered according to 2 four-dose schemes produced higher immunogenicity than YDV in three doses. If the finances and logistics allow it, a four-dose scheme and a full dosage of Engerix-B YDV seem preferable in countries where this vaccination scheme can easily be incorporated into the existing EPI programme (as in the Netherlands). Engerix-B YDV according to a four-dose scheme starting at month 3 yields a significantly higher immunogenicity than YDV in four doses starting at birth. This finding confirms results from our previous studies of 4 doses PDV in newborns of HBsAg-positive mothers (Schalm et al., 1989). This may be explained by the more mature immune system in children receiving their first vaccination later. Since we know that the efficacy of the combination of HBIg and hepatitis B vaccine is much greater than hepatitis B vaccine alone in preventing perinatal hepatitis B infection (Beasley et al., 1983), it is important that the children of HBsAg-positive mothers are covered with HBIg in the period between birth and active hepatitis B vaccination. In countries where HBIg is not sufficient and/or screening- programmes are not available, the decision to vaccinate against hepatitis B at birth or to wait and give the first dose together with other routine immunizations may only be taken after assesment of the risk to the infant of early infection, a risk which varies from region to region. Advancement of the starting date for other vaccinations (such as DTPP) in these countries, if future studies prove that this is feasible, might also contribute to a solution. For the greatest possible compliance, incorporation into the existing immunization programme is very important, despite the lack of a recommended optimal strategy for the administration of hepatitis B vaccine. Another obstacle to establishment of these recommendations is the wide variety of vaccination schemes of the EPI programme in different parts of the world, even in Europe. After the publication of a study, in which the immune response to YDV did not interfere with the immune response to either diphtheria and tetanus toxoids or oral polio vaccine (Giammanco et al., 1991), Italy started a compulsory hepatitis B vaccination programme for all newborns in May 1991. Recently health authorities opted for mass hepatitis B vaccination of all newborns in countries where the overall HBsAg carrier rate exceeds 1% and the HBV markers occur in 10 to 20 per cent of the population (as in Italy); screening and selective vaccination are recommended for countries with a HBsAg carrier rate below 0.5% and HBV markers in 5 to 10 per cent of the population (as in France and the Netherlands) (Goudeau et al., 1990). In the Netherlands, where

until now only the high-risk population is screened and vaccinated, the HBsAg carrier rate is estimated at 0.8% (Schalm et al., 1989) but in the present study the anti-HBs and anti-HBc positivity rates for HBsAg-negative mothers were between 15 and 30 per cent, much higher than expected. It should be noted however that the population investigated included a lot of immigrants and is not fully representative of the Dutch population (table 1). It will depend on the (political) strategy of the health authorities whether or not mass hepatitis B vaccination will be initiated. The costs of new vaccines such as the combination DTPP-hepatitis B vaccine, as well as the results of studies on long-term vaccine efficacy to determine if and when booster doses will be needed will play a role in these decisions. Perhaps increased consultation between health authorities in Europe will lead to a decrease in the number of existing vaccination schemes and thus open the way for recommendations that will lead to the eradication of hepatitis B.

Acknowledgement

We thank all staff members of the obstretics clinic, W.C.J. Hop, J. Boot and M. Bakker-Bendik for their excellent help in the establishment of this manuscript.

The study was designed in close cooperation with Dr. A. Safary and supported by SmithKline Beecham Biologicals, Rixensart, Belgium.

References

Anonymous (1989): Preventie Hepatitis B bij pasgeborenen. Geneeskundige Hoofdinspectie van de Volksgezondheid. GHI bulletin: September 1989, pp 1-36.

Beasley RP, Hwang LY, Lee GC, Lan C, Roan C, Huang F (1983): Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 2:1099-102.

Cadranel S, Zeghlache S, Fernandez S, Safary A, Andre F (1987): Vaccination of newborns of HBsAg-positive carrier mothers with a recombinant DNA hepatitis B vaccine. Postgraduate Medical Journal 63, Suppl 2:159-60.

Centers for Disease Control (1990): Protection against viral hepatitis. Recommendations of the Immunization Practices Advisory Committee. Morbidity and Mortality Weekly Report 39:80-93.

Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'Doye R, Diop-Mar I, Chiron JP (1986): Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 2:1143-6.

Damme Van P, Vranckx R, Safary A, Andre FE, Path FRC, Meheus A (1989): Protective efficacy of a recombinant deoxyribonucleic acid hepatitis B vaccine in institutionalized mentally handicapped clients. American Journal of Medicine 87, Suppl 3A:26S-9.

Ghendon Y (1990): WHO strategy for the global elimination of new cases of hepatitis B. Vaccine 8:S129-33.

Giammanco G, Volti SL, Mauro L, Bilancia GG, Salemi I, Barone P, Musumeci S (1991): Immune response to simultaneous administration of a recombinant DNA hepatitis B vaccine and multiple compulsory vaccines in infancy. Vaccine 9:747-50.

Goilav C, Prinsen H, Piot P (1990): Protective efficacy of a recombinant DNA vaccine against hepatitis B in male homosexuals: results at 36 months. Vaccine 8, Suppl:S50-2.

Goudeau A, Denis F, Mounier M, Dubois F, Klein J, Godefroy A, Ballet M, Mountij A (1987): Comparative multicentre study of the immunogenicity of different hepatitis B vaccines in healthy volunteers. Postgraduate Medical Journal 63, Suppl 2:125-8.

Goudeau A and the European Regional Study Group (1990): Epidemiology and eradication strategy for hepatitis B in Europe. Lancet 8, Suppl:S113-6.

Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF, Ostrow DG, O'Malley PM, Penley KA, Altman NL, Braff E, Shipman GF, Coleman PJ, Mandel EJ (1986): Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. The New England Journal of Medicine 315:209-14.

Jilg W, Schmidt M, Deinhardt F (1987): Persistence of specific antibodies after hepatitis B vaccination. Journal of Hepatology 6:201-7.

Peetermans JH (1987): Specifications and quality control of a yeast-derived hepatitis B vaccine. Postgraduate Medical Journal 63, Suppl 2:97-100.

Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG (1976): Design and analysis of randomized clinical trials requiring prolonged observation of each patient. British Journal of Cancer 34:585-612.

Poovorawan Y, Sanpavat S, Pongpunlert W, Chumdermpadetsuk S, Chitinand S, Sakulramrung R, Tannirundorn Y, Sentrakul P, Safary A (1990): Immunogenicity and protective efficacy of a yeast-derived hepatitis B vaccine in neonates. In: Coursaget P, Tong MJ (eds): "Progress in Hepatitis B Immunization." London, Paris: John Libbey Eurotext, pp 371-7.

Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff YW, Christiaens CML (1989): Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 83:1041-8.

Scheiermann N, Gesemann KM, Kreuzfelder E, Paar D (1987): Effects of a recombinant yeast-derived hepatitis B vaccine in healthy adults. Postgraduate Medical Journal 63, Suppl 2:115-9.

Scheiermann N, Gesemann M, Maurer C, Just M, Berger R (1990): Persistence of antibodies after immunization with a recombinant yeast-derived hepatitis B vaccine following two different schedules. Vaccine 8, Suppl:S44-6.

Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A (1981): A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. Hepatology 5: 377-85.

Wilde De M, Cabezon T, Harford N, Rutgers T, Simoen E, Van Wijnendaele F (1985): Production in yeast of hepatitis B surface antigen by r DNA technology. Developments in Biological Standardization 59:99-107.

Chapter 4

Immunogenicity of two different dosages (10 μg and 5 μg) of recombinant DNA hepatitis B vaccine in healthy neonates

Canho del R, Grosheide PM, Voogd-Schotanus M, Huisman WM, Heijtink RA, Schalm SW

Vaccine: submitted for publication

Abstract

The immunogenicity of one half (5 μg) and a full (10 μg) dosage of recombinant DNA yeast-derived hepatitis B vaccine (HB-Vax-DNA) in healthy neonates was assessed in order to compare two candidate dosages of vaccine. After randomization 174 newborns of HBsAg-negative mothers entered the study. Neonates received 4 doses of either 10 μg or 5 μg hepatitis B vaccine, according to the DTP-polio immunization schedule at months 3, 4, 5 and 11. No serious adverse reactions were observed; 15.5% of vaccinated newborns suffered mild transient local symptoms. The vaccine was highly immunogenic irrespective of dosage of vaccine; all infants developed anti-HBs levels ≥ 10 IU/L, 99% ≥ 100 IU/L.

Ten μg hepatitis B vaccine produced higher antibody levels than 5 μg hepatitis B vaccine after primary vaccination (first three doses) but not after booster vaccination (fourth dose) ($p = 0.06$ and 0.75, respectively). Both vaccine dosages can be recommended for incorporation in the Expanded Programme on Immunization in the Netherlands.

Keywords: hepatitis B vaccine, immunogenicity, neonates.

Introduction

The WHO strategy for the control of hepatitis B virus (HBV) infection and its sequelae, chronic hepatitis, cirrhosis and hepatocellular carcinoma, is mass vaccination of infants within the framework of the WHO Expanded Programme on Immunization (EPI) (1). In 1982 plasma-derived hepatitis B vaccine was licensed in the Netherlands and a vaccination programme to determine an effective and practical immunization schedule for the prevention of hepatitis B in neonates of HBsAg positive mothers was initiated (2). More than 95% of infants developed protective levels of anti-HBs after passive active immunization (2). The most effective and immunogenic schedule was achieved with 4 adult doses of vaccine (10 μg HB-Vax-DNA, Merck Sharp & Dohme, West Point, USA), starting at 3 months of age concomitant with DTP-polio immunization (2,3). Higher antibody levels were obtained after administration of 3 adult doses of 10 μg HB-Vax-DNA (GMT anti-HBs at month 12: 1236 IU/L) than after 3 infant doses of 5 μg HB-Vax-DNA (GMT anti-HBs at month 12: 510 IU/L), both starting directly after birth (3). An anti-HBs level ≥ 10 IU/L is assumed to be protective against HBV (4) and an anti-HBs level ≥ 100 IU/L ensures a long-lasting immunity (5). HB-Vax-DNA recombinant hepatitis B vaccine, manufactured by Merck Sharp & Dohme Laboratories, was licensed in the Netherlands in 1987. Until now, no studies on the administration of adult and infant doses of HBvax-DNA recombinant hepatitis B vaccine, administered at months 3, 4, 5 and 11 to neonates of HBsAg-negative mothers, have been performed. Therefore we compared the immunogenicity of 10 μg and 5 μg HB-Vax-DNA recombinant hepatitis B vaccine administered according to a four-dose schedule, starting at month 3.

Methods

Subjects and randomization

All pregnant women attending the prenatal clinic of the University Hospital Dijkzigt Rotterdam, the Netherlands, were screened at their first visit for the presence of HBsAg, as well as their blood group /rhesus factor and screening for syphilis. HBsAg-negative mothers were informed about the current hepatitis B immunization programme. Informed consent was obtained from the mother for the participation of her infant. Country of birth and age of the mother, gestational age, birthweight and sex of the child were registered on the parturition form. To be accepted into the study, newborns had to weigh at least 2000 grs at birth and have a 5-minute Apgar score of

7 or higher. In total 214 newborns were randomized into groups I and II, according to Peto (6). Forty children were not included in the study for the following reasons: secondary refusal of the parents (n=30), birthweight less than 2000 grs (n=4), Apgar score less than 7 (n=5), perinatal death (n=1). As a result, 86 neonates of group I and 88 of group II entered the study (intention to treat).

Vaccine and vaccination-schemes

The ad antigenic recombinant DNA yeast-derived hepatitis B vaccine was provided by Merck Sharp & Dohme (10 μg and 5μg HB-Vax-DNA, Westpoint, USA); 10 μg HBsAg were contained in 1.0 ml, 5 μg HBsAg in 0.5 ml, both in single dose vials (lot numbers 496750P, 557860P, 5806990S, 609540S, 651450S for 10 μg HB-Vax-DNA and 460201N, 460202N, 088011CH-B, 580700S, 623040S, 676710T for 5 μg HB-Vax-DNA). The production, quality control and physiochemical characteristics of HB-Vax-DNA have been described elsewhere (7). The vaccine, always stored at 2-8 °C, was administered into the quadriceps muscle by a physician. Parents were asked to record any local (soreness, redness, swelling) or systemic (fever > 37.5 °C, gastrointestinal symptoms) symptoms during three days following each injection of vaccine. Concomitant side-effects to be due to DTP-polio immunization given at the same time were excluded from analysis. The 86 neonates of group I received four doses of 10 μg vaccine and 88 neonates of group II received four doses of 5 μg vaccine. The vaccine was administered in both groups at months 3, 4, 5 (primary vaccination) and 11 (booster vaccination).

Blood tests and laboratory methods

Two ml of blood were drawn at month 0 (umbilical cord blood) and months 4, 6, 11 and 12 in both groups.

HBsAg, anti-HBc and anti-HBs were measured by radioimmunoassay (Ausria II, Corab, Ausab, Abbott Laboratories Chicago, USA). Anti-HBs is expressed in International Units/Litre (IU/L) after comparison with the WHO standard preparation (Central Laboratory of the Netherlands Red Cross Blood Transfusion Services, Amsterdam, the Netherlands).

Statistics

Statistical analysis was aplied on two data sets: data according intention-to-treat data and clean data (data of all children who entered the study and were vaccinated and assessed according to the protocol). Results between "intention to treat data" and "clean data" did not differ. The results of analysis of the clean data

Table 1. Distribution of the entry-variables according to vaccination groups I and II.

Group	I	II	P value
Number of infants (N)	86	88	
Median gestational age (weeks)	40	39	0.20*
Median birthweight (grams)	3323	3218	0.16*
Sex of child: (N)			0.76**
Males	51	50	
Females	35	38	
Anti-HBs positive (N)	11	13	0.83**
Anti-HBc positive (N)	10	1	1.00**
Median age mother (years)	26	29	0.20*
Country of birth mother: (N)			0.45***
Netherlands	28	23	
Mediterranean	12	17	
Surinam	29	25	
Asian	1	4	
Miscellaneous	16	19	

* Wilcoxon Ranksum test
** Fisher's exact test
*** Chi-square test

are presented below. Differences in discrete variables were analysed by Fisher's exact test and Chi-square test. Continuous variables were analysed by the two-sample Wilcoxon Rank sum test for unpaired observations. Limit for significance was set at 0.05. Geometric mean anti-HBs levels (GMT) are expressed in IU/L with 95% confidence intervals (CI).

Results

From April 1990 to March 1992, 174 infants born of HBsAg-negative mothers entered the study and were vaccinated according to the protocol. After primary

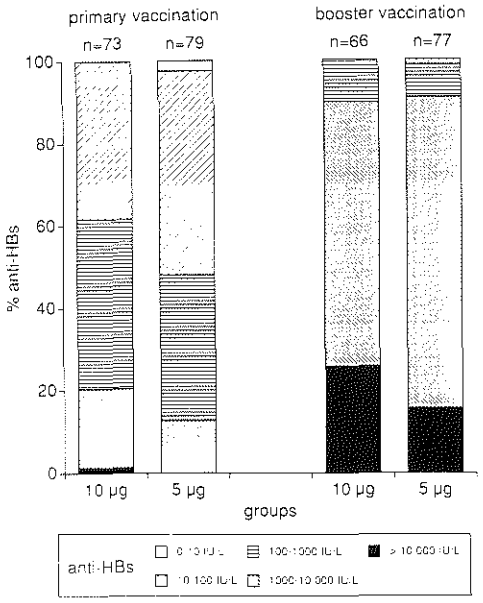

Figure 1. Percentage of infants with various anti-HBs levels after primary- and booster- vaccination with 10 µg and 5 µg HB-Vax-DNA hepatitis B vaccine administered at months 3, 4, 5 and 11 months of age.

vaccination serum samples were obtained in 73 (85%) and 79 (90%) cases in group I and II, respectively. After booster vaccination, 66 (77%) and 77 (88%) samples were available. There were no significant differences in country of birth of the mother, median age of the mother, median gestational age, median birthweight, sex of the child and number of infants with anti-HBs positivity and anti-HBc positivity in cord blood between the two groups (table 1).

Passive transfer of maternal antibodies

Passively acquired anti-HBs positivity rates for cord blood were 13% (n=11) and 15% (n=13) for groups I and II, respectively. Anti-HBc positivity rates at birth were 12% (n=-10) and 13% (n=11), respectively. In all but one case anti-HBc was negative at month 12 (in 1 case the test result was not available).

Anti-HBs seroconversion rate

Figure 1 shows the frequencies of protective levels of anti-HBs levels 1 month after primary and booster vaccination in the two groups. All infants who received 10 µg of vaccine (group I) developed protective anti-HBs levels (≥ 10 IU/L) after primary vaccination. All infants who received 5 µg of vaccine (group II) developed anti-HBs levels ≥ 10 IU/L, but in 2 cases this did not occur until after the booster vaccination. As far as long-term protection is concerned, 100% of infants in group I had anti-HBs levels ≥ 100 IU/L after the booster vaccination; for group II the corresponding percentage was 99%; one child had an anti-HBs level between 10 and 100 IU/L. Ultimately 88% of the infants in group I had anti-HBs levels ≥ 1000 IU/L after the booster vaccination in comparison to 90% of the infants in group II.

The anti-HBs level for 2 children in group II were 1 IU/L and 6 IU/L, respectively, after primary vaccination. After the booster vaccination these childeren exhibited 64 and 8078 IU/L, respectively.

Table 2 lists the anti-HBs levels (GMT) with 95% CI (anti-HBs > 0 IU/L) for the two different schedules. The GMT anti-HBs, obtained 1 month after primary vaccination and after booster vaccination with 4 doses of 10 μg HB-Vax-DNA were 231 IU/L and 4119 IU/L, respectively. This tends to be significantly (Wilcoxon test: p = 0.06) higher than those found for infants in group II after primary vaccination (GMT anti-HBs: 137 IU/L) but not (Wilcoxon test: p = 0.75) after booster vaccination (GMT anti-HBs: 3823 IU/L).

Anti-HBs levels at month 24 were available for 32 infants of group I (GMT anti-HBs 290 IU/L, 95% CI: 187-449) and 36 infants of group II (GMT anti-HBs 231 IU/L, 95% CI: 138-386).

No significant differences in GMT anti-HBs were found between boys and girls, presence of passively acquired maternal antibodies (anti-HBs, anti-HBc) and various countries of birth of the mother.

Adverse reactions to vaccine

No cases of clinical hepatitis or serious side-effects of the vaccine were reported. In 27 cases (15.5%) the parents of the vaccinated children reported transient red swelling at the site of injection or fever (less than 39 °C). No significant differences in the amount of side-effects between infants in group I and group II were found.

Table 2. Immunogenicity 1 month after primary and booster vaccinations with 10 μg and 5 μg HB-Vax-DNA hepatitis B vaccine administered at 3, 4, 5 and 11 months.

group	No	month	anti-HBs (> 0 IU/L) (GMT)	95% CI	P value
1 month after primary vaccination					
I (10 μg)	73	6	231	163-328	0.06[a]
II (5μg)	79	6	137	99- 190	
1 month after booster vaccination					
I (10 μg)	66	12	4119	3064-5538	0.75[a]
II (5 μg)	77	12	3823	3008-4859	

[a] Wilcoxon test scheme I versus II

Table 3. Immunogenicity of at least 3 doses of hepatitis B recombinant vaccine (HB-Vax-DNA, Merck Sharp & Dohme) in healthy neonates and infants.

Author (year public.)	Dose (μg HBsAg)	Scheme in months	No of vaccinees	Age-group (neonates mother HBsAg +/-)	Sero-conversion rate (%)*	GMT anti-HBs of converters (95 %CI)*
Lai ('86)	5	0, 1 and 6	21	infants neonates +	100[2]	1894 (?)
Stevens ('87)	5	0, 1 and 6	83	neonates +	100[6]	307
Milne ('88)	10	0, 1 and 6	56	infants	98[2]	3246 (2272-4636)
	5	0, 1 and 6	60		98[2]	2305 (1638-3158)
	2.5	0, 1 and 6	56		98[2]	1370 (866-2169)
Stevens ('90)	5	0, 1, 2 and 6	94	neonates +	96[3]	509
Goh ('92)	5	0, 1 and 6	31	infants	100[3]	1699 (?)
	2.5	0, 1 and 6	30		93[3]	1689 (?)
	1.25	0, 1 and 6	29		100[3]	1135 (?)
	0.6	0, 1 and 6	31		95[3]	1088 (?)
Canho del	10	3, 4, 5 and 11	65	neonates -	100[1]	4119 (3064-5538)
(present study)	5	3, 4, 5 and 11	71	neonates -	100[1]	3823 (3008-4859)

* 1 ([1]), 2 ([2]), 3([3]) or 6([6]) months after completion of vaccination scheme

Discussion

The 10 μg and 5 μg HB-Vax-DNA hepatitis B vaccines used in this study were safe and highly immunogenic. No serious side-effects were reported. In 15.5% of the infants mild transient local symptoms were present, irrespective of the dose of vaccine. This finding is in agreement with earlier results (8,9).

All infants reached anti-HBs levels ≥ 10 IU/L, 99% ≥ 100 IU/L, after completion of the vaccination schedule (figure 1). In table 3 the results of different studies on the immunogenicity of at least 3 doses of recombinant hepatitis B vaccine (MSD) in healthy neonates and infants are listed. Although there is accordance in the seroconversion rates, the GMT anti-HBs differs (median 1694 IU/L, range 307-4119 IU/L). These differences might be explained by differences

in maternal HBsAg status, vaccine, dose, schedule, time of blood sampling, age and ethnic background of the vaccinees.

In comparison with a full dosage (20 μg) of Engerix-B recombinant hepatitis B vaccine (SmithKline Beecham Biologicals) administered at months 3, 4, 5 and 11 to neonates of HBsAg-negative mothers (10), GMT anti-HBs at months 12 and 24 were 20768 IU/L and 2957 IU/L, respectively, 5 and 10 times higher than those obtained with 10 μg HB-Vax-DNA in the present study. The seroconversion rate for 20 μg Engerix-B recombinant vaccine (SKB) was the same as that for 10 μg HB-Vax-DNA recombinant vaccine (MSD), both being 100% (10). In another study four doses of 10 μg Engerix-B resulted in GMT anti-HBs of 2565 IU/L one month after the fourth dose, which is 1.5 times lower than in the present study; the seroconversion rate was 97.1% (11).

Our previous study (2) with 10 μg plasma vaccine (MSD), administrated at months 3,4,5 and 11, showed that plasma vaccine gives a significantly higher (Wilcoxon Rank-sum test $p = 0.0001$) anti-HBs response (n=99, peak level at month 12: 13427 IU/L) than 5 and 10 μg of the recombinant vaccine evaluated in the present study (n=143, peak level groups I and II at month 12: 3957 IU/L). This finding is in agreement with previous studies (12,13). The proportional decline in anti-HBs between 12 and 24 months was not significantly different (Wilcoxon Rank-sum test p=0.3572) between plasma vaccine (91%, 95% CI: 21-98) and recombinant vaccine (91%, 95% CI: 71-98). This is in agreement with the study of Jilg et al. (14), who found a 90% reduction in anti-HBs, 12 months after the last vaccination with 20 μg, 10 μg and 5 μg plasma vaccine. However more long-term immunogenicity studies on recombinant hepatitis B vaccine are needed to determine whether the decline of antibodies resembles that for plasma hepatitis B vaccine.

The recommended dosage of vaccine for neonates is ambiguous; Dutch authorities advise a full dosage of 10 μg HB-Vax-DNA (15); the Immunization Advisory Committee recommends half this standard adult dosage, 5 μg (16).

Recently, 2 μg and 2.5 μg doses of HB-Vax-DNA were recomended for the national childhood immunization programme because no significant differences in seroconversion rates could be found between 2.5 μg, 5 μg and 10 μg HB-Vax-DNA, administered at months 0, 1 and 6 to anti-HBs negative infants, after completion of the vaccination schedule (17,18). Much lower dosages of 0.6 μg and 1.25 μg HB-Vax-DNA were even as immunogenic as 2.5 μg, but not before month 9 (18). We found that 5 μg HB-Vax-DNA builds up a lower anti-HBs level than 10 μg HB-Vax-DNA after primary vaccination but that difference disappears after the booster vaccination.

The conclusion is that lower dosages of vaccine require more time to build up the same immunogenicity. For universal vaccination of infants of HBsAg-

negative mothers the rapidity of acquiring the required immunogenicity is less important than for infants of HBsAg-positive mothers, who are at greatest risk for HBV.

It appears that health authorities must opt for that vaccine dosage that will satisfy the task they set up.

Acknowledgement

We thank all staff members of the obstretics clinic, J. Boot and M. Bakker-Bendik for their excellent help in the establishment of this manuscript.

The study was designed in close cooperation with Dr. G.J. Gamelkoorn and H.J. Elgersma and supported by Merck Sharp & Dohme, Westpoint, United States of America.

References

1. World Health Organization, Expanded Programme on Immunization, Global Advisory Group. Weekley Epidemiological Record 1992; 3: 11-16.
2. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989; 83: 1041-7.
3. Grosheide PM, Schalm SW. Prevention of hepatitis B in neonates of HBsAg carrier mothers: comparison of 4 immunization schedules. In: Coursaget P, Tong MJ, eds. Progress in Hepatitis B Immunization: Colloque INSERM/ John Libbey Eurotext Ltd 1990; 194: 379.
4. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. Hepatology 1981; 5: 377-85.
5. Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986; 315: 209-14.
6. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J of Cancer 1976; 34: 585-612.
7. McAleer WJ, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. Nature 1984; 307: 178-80.
8. Dandolos E, Roumeliotou-Karayannis A, Richardson SC, Papaevangelou G. Safety and immunogenicity of a recombinant hepatitis B vaccine. J Med Virol 1985; 17: 57-62.
9. Milne A, Moyes CD, Allwood GK, Pearce NE, Krugman S. Antibody responses to recombinant, yeast-derived hepatitis B vaccine in teenage New Zealand children. NZ Med J 1988; 101: 67-9.
10. Canho del R, Grosheide PM, Voogd M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of a full dose (20 μg) of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes J Med Virol: 1993; 41: 30-34.
11. Poovorawan Y, Sanpavat S, Pongpunlert W, Chumdermpadetsuk S, Chitinand S, Sakulramrung R et al. Immunogenicity and protective efficacy of a yeast-derived hepatitis B vaccine in neonates. In: Coursaget P, Tong MJ (eds): `Progress in Hepatitis B Immunization'. London, Paris: John Libbey Eurotext 1990, pp 371-7.
12. Jilg W, Lorbeer B, Schmidt M, Wilske B, Zoulek G, Deinhardt F. Clinical evaluation of a recombinant hepatitis B vaccine. Lancet 1984; ii: 1174-5.
13. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. Jama 1987; 257: 2612-6.
14. Jilg W, Schmidt M, Deinhardt (F). Persistence of specific antibodies after hepatitis B vaccination. J Hepatol 1988; 6: 201-7.
15. Anonymous. Preventie hepatitis B bij pasgeborenen. Geneeskundige Hoofdinspectie van de Volksgezondheid. GHI bulletin September 1989, pp 1-36.
16. Centers for Disease Control. Protection against viral hepatitis. Recommendations of the Immunization Practices Advisory Committee. Morbidity and Mortality Weekly Report 1990; 39: 80-93.
17. Milne A, Brawner TA, Dumbill PC, Kawachi I, Pearce NE. Comparison of the immunogenicity of reduced doses of two recombinant DNA hepatitis B vaccines in New Zealand Children. J Med Virol 1989; 27: 264-7.
18. Goh KT, Tan KL, Kong KH, Oon CJ, Chan SH. Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: a four-year follow up study. Bulletin of the World Health Organization 1992; 70 (2): 233-9.

Chapter 5

Ten-year neonatal hepatitis B vaccination program, the Netherlands, 1982-1992: protective efficacy according to maternal serum levels of HBV-DNA and long-term immunogenicity.

Canho del R, Grosheide PM, Mazel JA, Heijtink RA, Hop WCJ, Gerards LJ, Gast de GC, Fetter WPF, Zwijnenberg J, Schalm SW and the Dutch study group Prevention Neonatal Hepatitis B

Submitted for publication

Abstract

From 1982-1989, 705 infants born to HBsAg positive mothers entered the Dutch neonatal hepatitis B vaccination program and received passive-active hepatitis B immunization, according to 6 schedules, varying in time of onset vaccination, dose of hepatitis B immunoglobulin (HBIg) and type and dose of vaccine. 118 (17%) of the mothers were also HBeAg positive. This final report describes the protective efficacy and long-term immunogenicity of passive-active hepatitis B immunization over a period of 10 years.

During follow up, 9 infants became HBsAg carriers; 8, all born to HBeAg positive mothers within the first year and another child, born to an HBeAg negative mother at the age of 5 years. No evidence of emergence of escape hepatitis B mutants was found. Protective Efficacy Rate (PER) of passive-active hepatitis B immunization at 12 months follow up was 92% for the total group with no significant differences between groups starting active immunization at birth or at 3 months; groups receiving one or two doses of HBIg or groups receiving plasma-derived or recombinant vaccine. The PER at month 12 in the group with maternal HBV-DNA levels less than 150 pg/ml was 100% and significantly higher than the 68% for the group with HBV-DNA levels above 150 pg/ml.

After 5 years of follow up, the group with active hepatitis B immunization starting at birth had significantly more infants with anti-HBs levels less than 10 IU/L (15%) than the corresponding group starting at 3 months (2%). Geometric Mean Titres of anti-HBs were significantly higher in the group, starting at 3 months of age with plasma vaccine than in the corresponding group receiving recombinant vaccine.

This program showed that passive-active hepatitis B vaccination can be highly effective in the prevention of neonatal hepatitis B, except for children born to women with high hepatitis B viraemia. Evaluation of vaccine schedules should taken into account risk assessment according to maternal HBV-DNA levels. The excellent efficacy of delayed active vaccination allows incorporation of hepatitis B vaccine into the standard infant immunization programs for countries with a passive-active immunization strategy for hepatitis B. For long-term protection, dosage of recombinant vaccine with equal immunogenicity to that of plasma vaccine should be considered.

Introduction

Passive-active immunization with hepatitis B immunoglobulin (HBIg) and hepatitis B vaccine has proven to be highly effective in preventing perinatal transmission of hepatitis B infection for more than a decade (1-3).

These studies were performed in infants born to HBeAg positive mothers, who are considered at nearly 90% risk for developing chronic hepatitis B virus infection (1, 4-6). Recently the risk of maternal to infant transmission of HBV was shown to be related to the presence of HBV-DNA in serum of HBeAg carrier mothers (7-9). The protective efficacy rate of passive-active hepatitis B immunization has not been assessed according to maternal HBV-DNA levels.

When hepatitis B vaccine was licensed in The Netherlands in 1982, a program was started to screen mothers for HBsAg positivity and to immunize their offspring. The vaccination program evaluated -in addition to various passive-active immunization schedules starting at birth- a schedule with passive immunization at birth and active immunization starting at 3 months of age, concomittant with Diphteria-Tetanus-Pertussis and Poliomyelitis (DTPP) vaccination. We hypothesized that efficacy of delayed active immunization would be similar, but long-term immunogenicity superior to immunization starting at birth. Such a result would allow incorporation of active hepatitis B immunization in the standard infant immunization program, which was thought to be advantageous with respect to logistics and costs. Preliminary results confirmed the hypothesis (10).

With the introduction of recombinant vaccine (1987), confirmation was also needed for recombinant vaccine (11). This study also allowed testing the need for supplementary hepatitis B immunoglobulins (HBIg) at the start of delayed active immunization.

Our findings that within the population studied, vaccination failure is primarily related to high maternal HBV-DNA levels (12) made it necessary to reevaluate the effects of components of the various schedules; time of starting active immunization, types and doses of HBIg and vaccine used, in relation to maternal HBV-DNA levels.

This final report of the Dutch program for prevention of perinatal hepatitis B describes the protective efficacy and long-term immunogenicity of passive-active hepatitis B immunization over a period of 10 years.

Subjects and methods

Hepatitis B screening

The study was started in July 1982 in three large city hospitals in Rotterdam and Utrecht and one large rural area, Twente-Gelderse Achterhoek. Blood samples

taken from all pregnant women at their first visit to the prenatal clinic of the participating centers were tested for the presence of HBsAg, in addition to the routine determination of blood group/rhesus factor and screening for syphilis. In case of a positive finding for HBsAg, a repeat HBsAg test was performed at 28 weeks of pregnancy. If HBsAg positivity was confirmed, randomization to one of the immunization schedules took place after informed consent was obtained from the mother. In the two participating hospitals in Rotterdam, the HBsAg status of expectant mothers was checked soon after arrival in the delivery room. Whenever prenatal HBsAg test results were missing, blood was obtained and tested the next morning with a rapid hemagglutination HBsAg test. If the rapid HBsAg test was positive, the mother was asked for informed consent and the baby was randomized and included in the immunization trial. All pregnant HBsAg positive women were also tested for the presence of HBeAg. In december 1992, maternal HBV-DNA levels were quantified retrospectively in the available stored serum samples positive for HBeAg.

Subjects and immunization schedules

From July 1982-January 1988, 495 eligible babies were randomly allocated to one of the four plasma-vaccine immunization schedules (table 1: groups I-IV); from January 1988-October 1989, another 210 eligible babies were allocated to one of the two recombinant-vaccine immunization schedules (table 1: groups V-VI).

All infants received HBIg (200-300 IU, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands) intramuscularly within two hours of birth by the physician or midwife in charge of the delivery. For active immunization, infants were referred to a pediatrician. Infants received plasma vaccine (10 μg and 5 μg HBvax, Merck Sharp & Dohme, West Point, USA) or recombinant vaccine (20 μg Engerix-B, SmithKline Beecham, Rixensart, Belgium). Nine infants (1 in group II, 3 in group III and 5 in group IV) with an anti-HBs level ≤ 10 IU/L at 12 months of age and a negative test for HBsAg received an additional course of plasma- or recombinant- vaccine in their second year of life (13).

Serological assays and laboratory methods

Blood samples were taken from the infants at birth (cord blood) and in groups I and II at months 3, 6, 11, 12, yearly until 9 years of age; in groups III and IV at months 3, 6, 12, yearly until 5 years of age; in groups V and VI at months 3, 4, 6, 11, 12 and at 2 years of age. All serum samples from the babies were tested for anti-HBs and anti-HBc; HBsAg was assayed in all samples with anti-HBs below 100 IU/L.

Table 1. Immunization schedules of study groups.

Groups	entry period	number of infants total (mother HBeAg +)	evaluated for immunogenicity	evaluated for efficacy[12 months]	HBIg[a] moths/dose after birth	vaccine type/dose	schedule months after birth
I	1982-1984*	117 (38)†	110 (35)	103 (37)	0/200	plasma[b] 10 μg	0, 1, 2 and 11
II	1982-1984	121 (42)	109 (37)	105 (41)	0,3/200,120	plasma 10 μg	3, 4, 5 and 11
III	1984-1987*	133 (3)	128 (2)	127 (3)	0/200	plasma 10 μg	0, 1, 6
IV	1984-1987	124 (4)	122 (4)	115 (2)	0/200	plasma 5 μg	0, 1, 6
V	1988-1989	112 (14)	102 (13)	98 (14)	0/300	recomb.[c] 20 μg	3, 4, 5 and 11
VI	1988-1989	98 (17)	93 (17)	83 (17)	0,3/300, 300	recomb. 20 μg	3, 4, 5 and 11
Total	1982-1989	705 (118)	664 (108)	631 (114)			

* Infants of HBeAg positive mothers entered in group I and II until december 1987
† Between parenthesis number of infants with HBeAg positive mothers
[a] Hepatitis B immunoglobulin,Central Laboratory of the Dutch Red Cross Blood Transfusion Service, Amsterdam, the Netherlands
[b] HBvax, Merck Sharp & Dohme, Westpoint, USA
[c] Engerix-B, SmithKline Beecham Biologicals, Rixensart, Belgium

HBsAg, HBeAg, anti-HBc and anti-HBs were assessed using a commercial radioimmunoassay kit (Abbott Laboratories, Chicago, ILL, USA), HBV-DNA quantitatively by a solution hybridization assay (HBV-DNA, Abbott Laboratories, USA).

Definition of HBV infection

A HBV carrier state was defined as being HBsAg positive for more than 6 months. Transient HBV infection was characterised by being HBsAg positive less than 6 months. Inapparent HBV infection was defined as anti-HBc positive without HBsAg on two or more occasions after months 12.

Statistical analysis

Data available were analyzed according to intention-to-treat principle. Separate per-protocol analysis, i.e. analysis of data of children who received vaccinations according to protocol, were also performed. The outcomes of these analyses did not significantly differ. Therefore we report on the protective efficacy using outcomes of the intention-to-treat analysis (including infants who violated the protocol); the results on the immunogenicity are reported using the outcomes of the per-protocol analysis.

Protection by different immunization schedules was expressed as the protective efficacy rate (PER) = 100% multiplied by (expected number of HBV infections without immunoprophylaxis minus measured number of HBV infections with immunoprophylaxis divided by the expected number of HBV infections without immunoprophylaxis). The expected number of HBV infections without immunoprophylaxis was estimated to be 90% of the number of infants from HBeAg positive mothers per immunization group (3-5). PER was also calculated for a percentage of 67 for expected number of HBV infections without immunoprophylaxis, in view of the finding that one third of HBeAg positive mothers of infants had a HBV-DNA level less than 5 pg/ml and therefore unlikely to transmit hepatitis B to their infants (8).

To investigate whether patients with incomplete data affected the outcomes of the long-term immunogenicity, the percentages of infants with anti-HBs levels <10 IU/L were also calculated for the group of infants which had no missing data (excluding occasional missing data, that could be obtained by linear interpolation of 2 adjacent points). The percentages thus obtained differed at all time-points at most 4% of those given in figure 3, indicating that loss to follow up did not significantly affect the outcomes as presented.

Differences in percentages were analysed by Chi-square test or Fisher's exact test in case of small numbers. Continuous variables were analysed by the two-sample Wilcoxon rank-sum test. Limit for significance was set to 0.05 (two-sided). In case of evaluations at various timepoints, the limit of significance was set according to Bonferoni's principle to allow for the multiplicity of statistical tests. Anti-HBs levels were expressed in IU/L. Geometric mean titers (GMT) were calculated only for those infants who had anti-HBs ≥ 10 IU/L. Exact confidence limits for odds-ratio were calculated using the statistical software package 'STAT-XACT'.

Ethics

The study was approved by the local Medical Ethics Committee of the participating centres.

Results

Follow up

From July 1982 until October 1989, 705 infants of HBsAg positive mothers were randomized; 16 infants were withdrawn after informed-consent but before vaccination, 12 infants received at least 1 vaccination, but no serum sample was available after month 0. 677 infants received passive-active immunization according to 6 schedules (table 1). Thirteen infants received vaccinations but not according to protocol (1 in group I, 4 in group II, 1 in group III, 1 in group IV, 3 in group V and 3 in group VI). 664 infants received passive-active immunization according the protocol, 650 of 664 infants (98%) received all planned vaccinations. Serum samples were available from 590 children at 1 year follow-up, 546 after 2 years, 262 after 5 years and 126 after 8 years (Table 4, appendix). Incomplete data from these children were primarily due to secondary refusal of the parents, related to the frequency of blood sampling (up to 10 samples) during the study, and migration.

Protective efficacy of perinatal HBV immunization

At month 12, HBsAg positivity was found in 8 of 590 infants. Of 33 infants no serum sample was available at month 12 but they were HBsAg negative thereafter, assuming no HBsAg positivity at month 12. From 8 out of 13 infants, who received a vaccination schedule not according to protocol, serum samples were available and found to be HBsAg negative at month 12 or thereafter. Together of

631 (590+33+8) infants analyzed, 8 (1.3%) were found to be HBsAg positive during the first year of life. All HBsAg positive children were born to HBeAg positive mothers. One infant of an HBeAg negative mother became HBsAg positive at the age of 5 years (figure 1). This child and all infants found to be HBsAg-positive in the first year, became hepatitis B carriers.

Inapparent HBV infection (anti-HBc positivity with negative HBsAg test on two or more occasions after month 12), was observed in 8 infants, all of HBeAg positive mothers. 614 (631-9-8) infants developed anti-HBs after vaccination, without signs of HBV infection; in 9 children anti-HBs more than 10 IU/L developed only after revaccination.

At month 12, Protective Efficacy Rate (PER) of passive-active immunization for the 114 infants (all immunization groups) of HBsAg and HBeAg positive mothers was 92% (Table 2). No significant differences were found between the PER of infected infants in the groups starting active vaccination at birth and groups starting at 3 months; between groups receiving 1 dose of HBIg and groups receiving 2 doses of HBIg and between groups receiving plasma- and those receiving recombinant vaccine (table 2).

In 72 of the 114 HBeAg positive mothers, residual serum was available for quantitative HBV-DNA assessment in 1992. Table 3 shows the relation of maternal

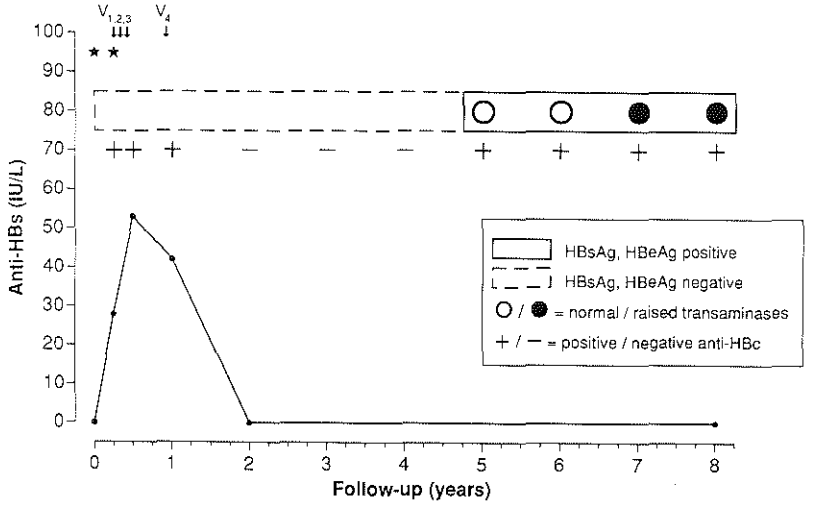

Figure 1. *Chronic HBV infection after 5 years of follow up in one infant, born to an HBeAg negative mother, despite a weak response to neonatal passive-active hepatitis B immunization (group II).*

Analysis of serum samples of family members showed that this patient has an HBeAg positive brother. This might explain the horizontally transmitted HBV infection in this patient at the age of 5 years. There was no coexistence of HBsAg and anti-HBs positivity in the patient, full in vitro neutralization of HBsAg by HBIg was observed, indicating that the HBV infection was not caused by an escape mutant.

HBV-DNA levels (divided in 3 groups of equal size) and number of HBV carrier infants. The PER at month 12 for the 2 groups with HBV-DNA levels < 150 pg/ml were 100% and 32% higher than that for the group with HBV-DNA levels ≥ 150 pg/ml (P-value = 0.009).

Figure 2 shows the maternal HBV-DNA levels of HBV infected infants and of non-infected infants. Median maternal HBV-DNA of HBV carrier infants and of inapparently infected infants were ten times higher (approximatively 350 pg/ml) than median maternal HBV-DNA of infants without HBV infection (31 pg/ml) (p= 0.001 and 0.03, respectively).

Table 2. *Efficacy of passive active hepatitis B immunization, at month 12 in infants of HBeAg positive mothers, according different vaccination methods*

Group	number of infants		HBsAg positivity difference in %	PER[12 months a] %	
	total	HBsAg positive (%)	(95 % CI)	expected % of infant HBV infections without prophylaxis	
				90 %	67 %
HBIg month 0	56	4 (7.1)	0.2 (-9.2, +9.2)	92	89
HBIg months 0,3	58	4 (6.9)		92	90
Vaccine starting month 0	42	3 (7.1)	0.2 (-9.5, +9.9)	92	89
Vaccine starting month 3	72	5 (6.9)		92	90
Plasma vaccine	83	6 (7.2)	0.7 (-9.6, +11.0)	92	89
Recombinant vaccine	31	2 (6.5)		93	90
Total	114	8 (7.0)		92	90

[a] PER= ((expected number of HBV infections without immunoprophylaxis minus measured number of HBV infections in immunization group) divided by the expected number of HBV infections without immunoprophylaxis) x 100%.

The expected number of HBV infections without immunoprophylaxis for infants from HBeAg positive mothers is usually estimated 90%. (3-5). PER was also calculated for 67 expected percentage of HBV infections without immunoprophylaxis, in view of the finding that one third of infants of HBeAg positive mothers had HBV-DNA less than 5 pg/ml and therefore unlikely to transmit hepatitis B to their infants (8).

Long-term immunogenicity

Assuming a minimal risk for hepatitis B infection with anti-HBs levels above 10 IU/L, and a potential risk with anti-HBs levels less than 10 IU/L (14), we calculated the percentages of infants with anti-HBs < 10 IU/L, in the different immunization groups (figure 3). At the age of 5 years, the immunization group starting at 3 months of age with plasma vaccine (group II) had a significantly lower percentage (2%, 95% CI: 0-6%) of children with anti-HBs < 10 IU/L than the immunization group starting at birth (group I), 14.5% (95% CI: 6-23%), (Fisher exact test p=0.02). The percentage of infants with anti-HBs < 10 IU/L in group II never exceeded 5% during 5-year follow up, whereas this corresponding percentage in the other immunization groups increased during follow up to more than 15%.

Total follow up of infants with anti-HBs less than 10 IU/L amounted to 186 person-years: 38 from 19 of 441 infants ≥ 1000 IU/L at month 12; 78 from 30 of 97 infants with anti-HBs between 100 and 1000 IU/L; 69 from 21 of 35 infants with anti-HBs between 10 and 100 IU/L and 1 from 1 of 9 infants with anti-HBs less than 10 IU/L (the latter all developped more than 10 IU/L after revaccination).

Six of the 8 infants with inapparent HBV infection had anti-HBs ≥ 1000 IU/L at month 12, 2 between 10 and 100 IU/L.

Table 4 shows the GMT of anti-HBs (anti-HBs >= 10 IU/L) in the different immunization groups during follow up. The GMT of anti-HBs of group II (10 μg plasma vaccine administration from 3 months of age onwards) was approximatively two times higher than the GMT of anti-HBs of group I (starting at birth with 10 μg plasma vaccine). In the groups with different dosages of vaccine, group III with 3 doses of 10 μg plasma vaccine administered from birth onwards, showed approximatively two times higher GMT anti-HBs at month 12 than group IV, with the corresponding schedule but only 5 μg of plasma vaccine. However this difference was not significant after 36 months of follow up. Comparison of plasma- and recombinant- vaccine administered from 3 months onwards showed, that the group with plasma vaccine (group II) had an approximately one and half times higher GMT of anti-HBs than the corresponding group with recombinant vaccine (group VI) (P-value < 0.001 at all times measured).

Side-effects

No clinically important side-effects of vaccination were reported by the parents or observed by the pediatricians.

Table 3. Protective Efficacy Rate (PER) of passive-active hepatitis B immunization at month 12 according to maternal HBV-DNA levels.

HBV-DNA (pg/ml)	number of infants: total	number of infants: HBsAg positive (%)	PER[12 months] 90 %	P-value
<6	24	0 (0)	n.a.[a]	
7-150	24	0 (0)	100	
>150	24	7 (29)	68	0.009*

* Fisher exact test between number of HBsAg positive infants in group with maternal HBV-DNA > 150 pg/ml and the groups with HBV-DNA between 7-150 pg/ml and < 6 pg/ml, respectively. Due to small numbers of infants, we calculated the exact 95% CI of the ratio of the odds of HBsAg positivity in the group of infants with HBV DNA < 150 pg/ml (odds = 0/48) versus the corresponding odds (= 7/17) in the group with HBV DNA > 150 pg/ml. This 95% CI ranged from 0 to 0.22.

[a] n.a. is not applicable, in view of the finding that one third of infants of HBeAg positive mothers had HBV-DNA less than 5 pg/ml and therefore unlikely to transmit hepatitis B to their infants (8).

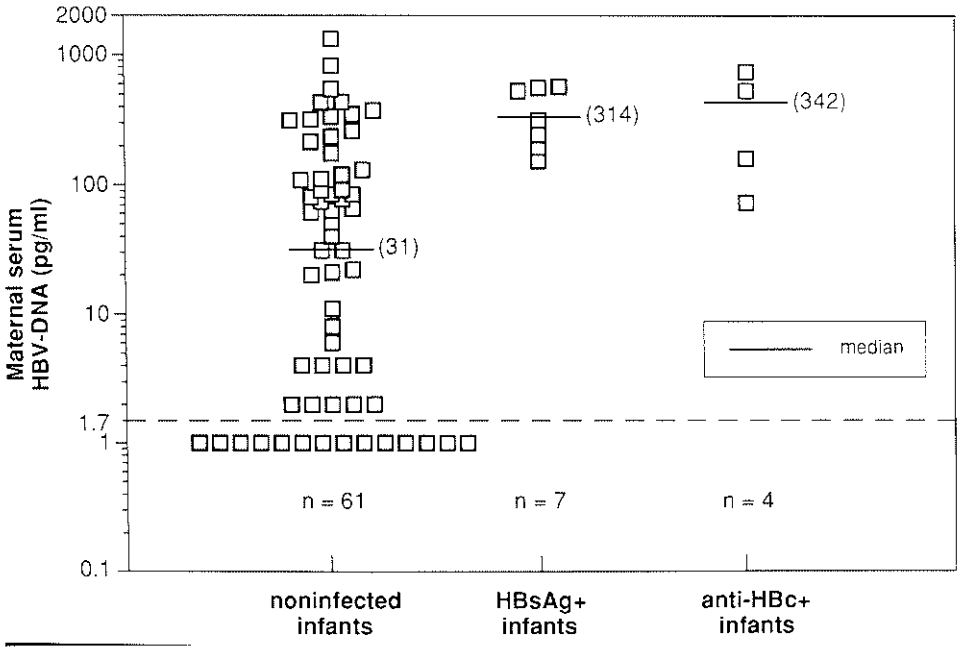

Figure 2. Maternal HBV-DNA levels of 7 persistent HBV infected infants, 4 transient HBV infected infants and of 61 noninfected infected infants. Cut-off level of HBV-DNA assay (HBV-DNA, Abbott, Ill, USA) is 1.7 pg/ml.

Maternal HBV-DNA was not available for 1 HBsAg positive infant, 4 anti-HBc positive infants and 37 noninfected infants of HBeAg positive mothers.

Discussion

In this study, the Protective Efficacy Rate at 12 months against hepatitis B infection for infants of HBeAg positive carrier mothers was 92%; for infants of HBeAg negative mothers it was 100%. These rates are comparable to those found in other passive-active immunization studies, with either plasma-derived or recombinant vaccine (1-3,15,16). During follow up beyond 1 year, extending to 9 years, one hepatitis B vaccinated infant of an HBeAg negative mother became positive for HBsAg.

There was no effect on PER for the timing of active immunization, the number of doses of HBIg and the type of vaccine. Although confidence in the results is not absolute, these results are confirming to earlier findings. Beasley et al. (1) already reported that, with HBIg coverage at birth, the timing of the start of active vaccination appeared of no importance. Stevens et al. (3) published results indicating that yeast-recombinant vaccine was as effective as plasma-derived vaccine in preventing hepatitis B virus infection. We found no evidence for a need for a second dose of HBIg in combination with delayed active immunization; evidence for the

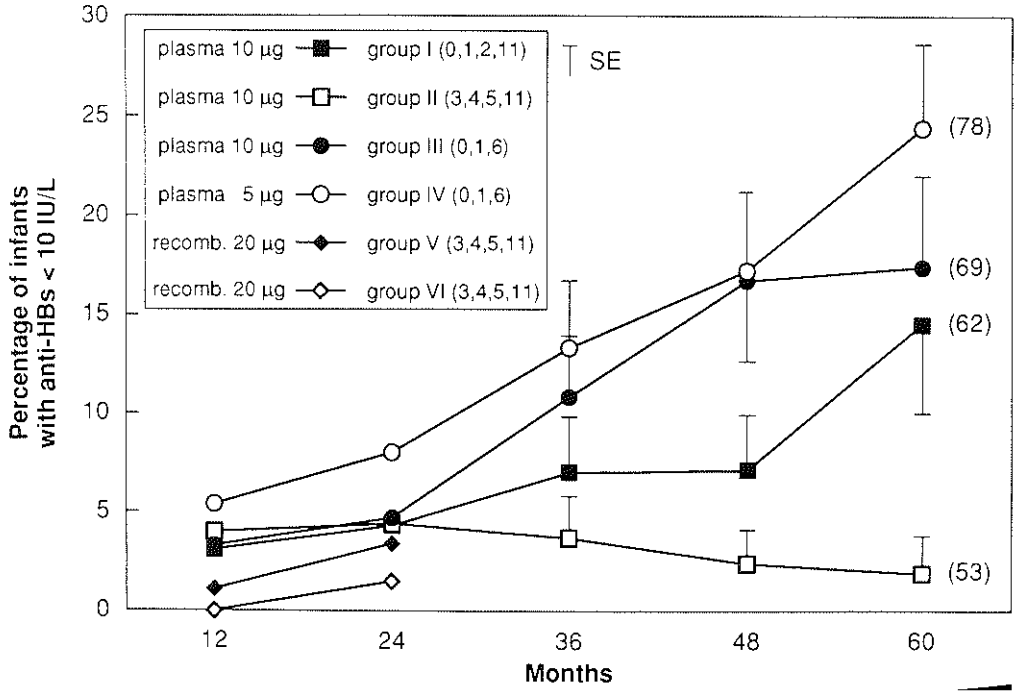

Figure 3. Percentages of infants with anti-HBs < 10 IU/L in the 6 immunization groups. Bars indicate standard errors.

Table 4. GMT (anti-HBs >=10 IU/L) in the 6 immunization groups during follow up

	GMT anti-HBs (95% CI) IU/L				
	months				
Groups	12	24	36	48	60
I 0, 1, 2, 11; 10 µg pl.	8730 (6238-12217)	737 (533-1019)	353 (248-502)	207 (145-296)	137 (92-203)
II 3, 4, 5, 11; 10 µg pl.	15739 (11738-21104)	1728 (1284-2325)	820 (596-1129)	484 (356-231)	331 (231-473)
P-value*	0.023	0.001	0.0006	0.0004	0.0002
III 0, 1, 6; 10 µg pl.	1142 (849-1537)	331 (245-447)	202 (151-271)	138 (100-90)	100 (70-143)
IV 0, 1, 6; 5 µg pl.	608 (438-846)	203 (146-283)	163 (116-228)	114 (78-166)	75 (52-109)
p-value**	0.0012	0.0066	0.19	0.31	0.11
V 3, 4, 5, 11; 20 µg rec.	9317 (6558-13237)	1727 (1216-2452)	-	-	-
VI 3, 4, 5, 11; 20 µg rec.	9699 (6475-14528)	1125 (767-1649)	-	-	-
P-value***	0.13	0.84			

* Between group I and II ** Between group III and IV *** Between group V and VI

Appendix

	number of serum samples available during follow up (%)									
	months									
groups	3	6	11	12	24	36	48	60	72	84
I	102	106	99	98	92	86	85	62	73	67
II	100	107	98	99	91	82	84	53	66	59
III	115	119	-	122	107	102	84	69	-	-
IV	114	113	-	112	100	98	87	78	-	-
V	94	91	91	90	88	-	-	-	-	-
VI	73	6	65	69	68	-	-	-	-	-
Total	598	542	353	590	546	368	340	262	139	126

necessity of such action has not been forthcoming. Although some uncertainty on this point may persist, we found a major clinically relevant factor that influenced the PER.

PER in the groups of infants with maternal HBV-DNA levels less than 150 pg/ml was 100% and significantly higher than 68% in the group with maternal HBV-DNA level of more than 150 pg/ml.

To verify the finding that the PER is markedly influenced by maternal HBV-DNA levels, we analyzed the protective efficacy rate at 12 months of age according to quantified maternal HBV-DNA levels, in another large neonatal hepatitis B vaccination program (8) (P.N. Lelie, written communication). These results strongly support the concept that the level of maternal HBV-DNA is the major factor influencing PER of hepatitis B immunization. In the Hong Kong study, no persistent HBsAg positivity at 12 months was detected in infants with maternal HBV-DNA below 6 pg/ml, irrespective of immunization. Infants with maternal HBV-DNA levels of more than 150 pg/ml were at high risk for hepatitis B (25-50% of infants became persistent HBsAg positive), despite immunization. In the Hong Kong study, infants with maternal HBV-DNA between 7-150 pg/ml were at risk for hepatitis B infection (15-28%).

The absence of HBsAg carriers in the group with moderately high maternal HBV-DNA in the Dutch study, in comparison to 15%-28% carrier rate in the Hong Kong study needs additional discussion. Since the rate of intrauterine infection is estimated to be only 1-2% (15), the difference observed is likely due to differences in intervention (HBIg, vaccine) or to differences in maternal fetal transfusion during labour. The dose of hepatitis B immunoglobulin used in the Hong Kong study (50 IU) was lower than that in the Dutch study (200-300 IU) and the dose of vaccine in the Hong Kong study (3 μg) was also lower than in the Dutch study (10 μg). Although the dose of vaccine may not reflect immunogenicity, the GMT of anti-HBs in the Dutch study were almost 10 times higher than in Hong Kong, indicating higher immunogenicity of the vaccine used in the study in the Netherlands. These results also suggest that efficacy of dose of HBIg and vaccine can now assessed more precisely in cohorts with maternal quantified HBV DNA levels.

Recently, hepatitis B "escape mutants", lacking the "a" epitope on the viral envelope were found in vaccinated infants (17). We did not observe coexistence of HBsAg and anti-HBs in 7 infants with persistent HBsAg. Additional laboratory investigations including in vitro neutralization of HBsAg by polyclonal anti-HBs have provided no evidence of the presence of surface antigen variants in our HBsAg positive children (12). Also a clinical relevance of precore mutants was not found in our study, since the PER at month 12 for infants of HBeAg negative mothers was 100%.

Long-term immunogenicity was significantly higher in the group with late active immunization than in the group starting directly after birth. At the age of 5 years, the group with delayed active immunization had a significantly lower percentage (2%, 95% CI: 0-6%) of children with anti-HBs less than 10 IU/L than in the groups starting at birth (15-25%). This finding is in agreement with others who found an enhancement of response if the infant was older at the time of the initial injection, probably related to a more mature immune system (18). The implications of these findings are at present unclear but may become of potential importance if more results of long-term follow up studies become available. If protection against hepatitis B infection in a major way depends on the degree of immunologic priming reflected by persistence of antibody, then a strong argument could be made for adoption of schedules that maximize anti-HBs levels. In the present study, there was a total follow up of 186 years with anti-HBs less than 10 IU/L in 71 infants. One infant, born to a HBeAg-negative mother, with an initial response between 10 and 100 IU/L became HBsAg carrier after 4 years of follow up without detectable anti-HBs. In other studies, no HBsAg positivity after 5 years of follow up was found for infants with initial anti-HBs ≥ 10 IU/L, whether the infants lost their anti-HBs or not (18,19). In long-follow up studies of immunised adults, no persistent HBsAg positivity was detected in persons with an initial anti-HBs response >10 SRU (14), but transient HBsAg positivity and/or anti-HBc positivity was detected in this group (less than 1% in 100 person-years exposed). For the time being, until more long-term follow up studies are available, it seems advisable to aim for an initial anti-HBs response of more than 10-100 IU/L for the prevention of clinically important forms of hepatitis B (20).

The implications of the finding that there was no effect on PER of the timing of active immunization, allows incorporation of hepatitis B vaccine into the existing Expanded Programme on Immunization (EPI). The number of injections and the number of doctor visits can then be reduced if multivalent vaccine becomes available. The finding that maternal HBV-DNA is the most relevant clinical factor influencing the efficacy of standard passive-active hepatitis B immunization is important for improving results of intervention, in particular in countries which can afford high level individual care. In practice, we advocate to assess HBV-DNA quantatively in all HBsAg positive mothers found to be also HBeAg positive. On the basis of the current study we advise to offer additional preventive measures for infants at high risk for hepatitis B: 300 IU of HBIg (1 ml instead of 0.5 ml) and adult dose of vaccine instead of pediatric dose to neonates of HBeAg positive mothers with maternal HBV-DNA level above 5 pg/ml. Further research is warranted to prevent hepatitis B in infants with maternal HBV DNA level above 150 pg/ml, like evaluation of delivery by Caesarean section as proposed by Lee (21), or additional HBIg injections as suggested by the Hong Kong study (8).

Acknowledgements

We thank M. Bakker-Bendik, Th. van der Laar-Kokx, H. Ribbers-Smeenk, H.C. van Dijk and M. Voogd-Schotanus for their outstanding help in this study for several years. Special thanks go to Y.A.M. Weber, J. Kruining for performing the laboratory tests, J. Boot for his technical assistance and P.N. Lelie for making available the maternal HBV-DNA assays of the Hong Kong neonatal hepatitis B vaccination program.

The following participants of the Dutch study group Prevention Neonatal Hepatitis B are acknowledged for their contribution:

Wladimiroff JW, Dept. of Obstetics, Academic Hospital Dijkzigt, Rotterdam; Nuijten ASM, State School of Training for Obstetricians, Rotterdam; Zwijnenberg J, Dept. of Pediatrics, Ruwaard van Putten Hospital, Rotterdam; Bänffer JRJ, Regional Public Health Laboratory, Rotterdam; Botman MJ, Regional Laboratory of Pathology and Microbiology, Enschede; Christiaens GCML, Dept. of Gynaecology and Obstetrics, Academic Hospital, Utrecht; Fetter WPF, Dept. of Pediatrics, St. Sophia Hospital, Zwolle; Baerts W, Dept. of Pediatrics, St. Sophia Hospital, Zwolle.

The study group is grateful to the Steering Committee formed by: Professor Dr. J. Huisman, Professor Dr. J.W. Stoop, Dr. H. Bijkerk and Professor S. Krugman.

This study was supported by a grant from the Prevention Fund, the Netherlands (no. 28-760-2)

References

1. Beasley RP, Hwang LY, Lee GC, Lan C, Roan C, Huang F et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-102.
2. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'doye R et al. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986; ii: 1143-5.
3. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine: Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. Jama 1987; 257: 2612-16.
4. Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y, Mayumi M. e Antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976; 294: 746-9.
5. Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJTh, Kalkhoven-Benschop J, Brummelhuis HGJ. Prevention of chronic HBsAg-carrier state in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. Lancet 1979; i: 436-7.
6. Beasley RP, Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Inf Dis 1983; 147: 185-90.
7. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP et al. Prevention of maternal-infant hepatitis B virus transmission by immunization: The role of serum hepatitis B virus DNA. Hepatol 1986; 6: 369-73.
8. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989; i: 406-10.
9. Lin HH, Chang MH, Chen DS, Sung JL, Hong KH, Young YC et al. Early predictor of the efficacy of immunoprophylaxis against perinatal hepatitis B transmission: analysis of prophylaxis failure. Vaccine 1991;9: 457-60.
10. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989; 83: 1041-8.
11. Grosheide PM, Canho del R, Voogd M, Heijtink RA, Schalm SW, Dutch Study Group Prevention Neonatal Hepatitis B. Anti-HBs levels in infants of hepatitis B carrier mothers after delayed active immunization with recombinant vaccine concomittant with DTP-Polio vaccine: Is there need for a second dose of HBIg? Submitted for publication.
12. Canho del R, Grosheide PM, Schalm SW, Vries de RRP, Heijtink RA. Failure of neonatal hepatitis B vaccination: The role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates. J Hepatol: in press.
13. Canho del R, Schalm SW, Heijtink RA. Hepatitis B revaccination of neonates with inadequate response after primovaccination. Vaccine 1992; 10: 69.
14. Hadler SC, Francis DP, James DS, Maynard JE, Thompson SE, Judson FN et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986; 315: 209-14.
15. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY et al. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984; i: 921-6.
16. Poovorawan Y, Sanpavat S, Pongpuniert W, Chumdermpadetsuk S, Sentrakul P, Safary A. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen-positive mothers. JAMA 1989; 261: 3278-81.
17. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E et al. Vaccine-induced escape mutant of hepatitis B virus. Lancet 1990; 336: 325-9.
18. Stevens CE, Toy PT, Taylor PE, Lee T, Yip HY. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long-term protection. Pediatr 1992; 90: 170-3.

19. Lo KJ, Lee SD, Tsai YT, Wu TC, Chan CY, Chen GH et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in infants born to HBeAg-positive HBsAg-carrier mothers. Hepatology 1988; 8: 1647-50.
20. Jilg W, Schmidt M, Deinhardt F. Decline of anti-HBs after hepatitis B vaccination and timing of revaccination. Lancet 1990; i: 173-4.
21. Lee SD, Lo KJ, Tsai YT, Wu JC, Wu TC, Yang ZL et al. Role of Caesarean section in prevention of mother-infant transmission of hepatitis B virus. Lancet 1988; ii: 833-4.

Chapter 6

Hepatitis B vaccination and preterm infants

Canho del R, Grosheide PM, Gerards LJ, Heijtink RA, Schalm SW

Dutch version of this manuscript: Tijdschrift Kindergeneeskunde 1992; 60: 105-11
(Short version of this manuscript: The Pediatric Infectious Disease Journal 1993; 5: 407-8)

Summary

Since 1986 Health Authorities in the Netherlands recommend vaccinating prematurely born infants at the appropriate chronological age, without correcting for their shortened gestational age as the practice used to be. The advise is based on research indicating that the immune response after DPT (Diphteria, Pertussis, Tetanus) vaccination of preterm infants is comparable to the immune response of full term infants. To see whether this also applies to hepatitis B vaccination, we examined the anti-HBs titers after hepatitis B vaccination of 44 preterm infants and compared these to the anti-HBs titers of 829 full term infants. Over 95% of the preterm infants developed an adequate immune response (> 10 IU/liter) after primary vaccination, irrespective of the vaccination schedule. The percentage of children with anti-HBs titers > 10 IU/liter was not different for the group of preterm infants from that of the group of full term infants (98%). Nor was it different when a value of > 100 IU/liter was considered. Differentiating between early and late active immunization we found that the percentage of children with anti-HBs titers > 10 IU/liter from the group vaccinated immediately after birth (98%) did not differ significantly from that from the group first vaccinated 3 months after birth (99%). There was, however, a signifficant difference between these two groups when a value of > 100 IU/liter was considered. This study lends support to the Health Authorities' advice to start vaccination of preterm infants at the appropriate chronological age, without correction for their shortened gestational age.

Introduction

Passive and active immunization of infants born to hepatitis B virus carriers is an effective and safe method for the prevention of perinatal transmission of the hepatitis B virus. In healthy, full term newborns an adequate immune response (antibodies against the surface antigen (anti-HBs) > 10 international units per liter (IU/liter) is found after immunization with hepatitis B vaccine according to various immunization schedules (1,2). Apart from administration method, dosage, and vaccination schedule, children's immune response to hepatitis B vaccination probably depends on several factors, including HLA type (3), the mother's country of origin (4), and the occurrence of diseases influencing the immune system (5,6). However, so far no data were available on the immune response of preterm infants after hepatitis B vaccination (7). In 1986 the Health Authorities recommended starting the vaccination of preterm infants at the appropriate chronological age rather than making allowances for their shortened gestational age, as the practice had been (8). This advice was based on a study showing the immune response of 25 preterm infants with a median gestation age of 31 weeks (range 28-34 weeks) to be similar to the immune response of full term infants after the second diphteria-tetanus-pertussis vaccination at chronological age (9). The question is whether preterm infants also develop an adequate immune response after hepatitis B vaccination at chronological age, that is, without correcting for their shortened gestational age. This article describes the immune response of preterm infants at 1-2 months after primary active-passive hepatitis B immunization (i.e. after the first 2 or 3 successive vaccinations, depending on the schedule), which response is then compared with the response of full term infants.

Patients and methods

From 1982 till 1992, 1025 neonates were vaccinated with hepatitis B vaccine at three centres in the Netherlands (Twente, Utrecht, Rotterdam); 705 children of hepatitis B surface antigen (HBsAg)-positive mothers received hepatitis B immunoglobulin (HBIg) at birth, followed by active immunization with plasma vaccine or recombinant vaccine, according to various schedules (Table 1). The vaccine was kept at a temperature of 2-6 °C and was injected in the quadriceps muscle. Blood sampling at birth was from the umbilical cord, and afterwards at 1, 3, 4, 6, 11 and 12 months, depending on the schedule, and followed by annual sampling during a median period of 5 years (range 2 to 8 years). HBsAg and anti-HBs levels in the serum were measured with radio-immunological tests (Ausria, Ausab, Abbott Laboratories, Chicago, Ill, USA). The gestational age of 46 of the

1025 children was less than 37 weeks (median 36.0 weeks; range 27.2 to 36.6 weeks), the age being determined on the basis of data from their birth reports. Records were kept of the mother's country of origin, of the gender, weight, apgar score and any perinatal problems for all preterm infants. Like the full term infants, all 46 preterm infants were vaccinated with hepatitis B vaccine according to various schedules, without correction for their premature births; 23 preterm infants were vaccinated with hepatitis B vaccine immediately after birth (16 of those children also received HBIg). The other 23 preterm infants started vaccination at 3 months (of those 13 received HBIg at birth) (Table 1).

Statistics

The immune responses of preterm infants after hepatitis B vaccination at their chronological age were compared with the immune responses of full term infants vaccinated according to corresponding schedules. Significance of percentage differences between groups was determined with the use of Fisher's exact test, with a threshold of 0.05 (both ways).

Results

Forty-six children with a gestational age of less than 37 weeks were vaccinated with hepatitis B vaccine (4.5% of the total number of children vaccinated). Of those 28 were boys and 18 were girls; their median weight at birth was 2540 grams (range 1200 to 3950 grams). For 10 preterm infants their birth weight was too low for their gestational age. 31 Preterm infants had an apgar score of 10 after 5 minutes, 15 had an apgar score <10 after 5 minutes (range 6 to 9). The countries of origin of the mothers of the preterm infants were similar to those of the mothers of the full term infants. Four of the 46 preterm infants needed ventilative support because of a hyaline membrane disorder and 2 of these received 2 and 4 exchange transfusions respectively because of hyperbilirubinemia; they received no extra HBIg. The infant with the shortest gestational age (27.7 weeks) suffered an intracranial haemorrhage and needed completely parenteral feeding for a number of weeks.

Serological response

Of the 46 preterm infants 44 received the first 2 or 3 (depending on the schedule) hepatitis B vaccinations and from each of these 44 infants at least one

Table 1. 46 preterm and 979 full term infants vaccinated with hepatitis B vaccine in three centers in the Netherlands, 1982-1992.

group	vaccination schedule month	serum sample month	HBIg *	HBvaccine **	preterm N	fullterm N
1	**0,1,2**,11	0,**3**,6,11,12	+	plasma 10 µg	4	113
2	**3,4,5**,11	0,3,**6**,11,12	++	plasma 10µg	5	116
3	**0,1**,6	0,**3**,6,12	+	plasma 10µg	5	128
4	**0,1**,6	0,**3**,6,12	+	plasma 5µg	7	117
5	**3,4,5**,11	0,3,4,**6**,11,12	+	recomb. 20µg	4	108
6	**3,4,5**,11	0,3,4,**6**,11,12	++	recomb. 20µg	4	94
7	**3,4,5**,11	0,4,**6**,11,12	-	recomb. 20µg	2	49
8	**0,1**,6	0,**3**,6,7,12	-	recomb. 20µg	4	52
9	**0,1,2**,11	0,**3**,6,11,12	-	recomb. 20µg	3	52
10	**3,4,5**,11	0,4,**6**,11,12	-	recomb. 10µg	2	76
11	**3,4,5**,11	0,4,**6**,11,12	-	recomb. 5µg	6	74
total					46	979

* 300 IU/ml hepatitis B immunoglobuline, (Centraal Laboratorium Bloedtransfusiedienst, Amsterdam). (+: HBIg month 0), ++: HBIg months 0 and 3),
** 10 µg plasma vaccine and 10 µg and 5 µg recombinant vaccine (Merck, Sharp en Dohme, Westpoint Pa, U.S.A.).
20 µg recombinant vaccine (SmithKline Beecham, Rixensart, Belgium)

The primary vaccinations and the month of serum sampling after these 2 vaccinations (schedule 3, 4 and 8) and after these 3 vaccinations (schedule 1, 2, 5, 6, 7, 9, 10 en 11) are in bold type.

serum sample was available after completion of the primary vaccination series. Of 2 preterm infants only one serum sample was known after the first vaccination; these children were excluded from further analysis. From 829 of the 979 full term infants serum samples were collected for analysis (primary vaccination was incomplete for 44 children and of 106 children no serum sample was available). Of the preterm infants 98% (43/44) had anti-HBs titers > 10 IU/liter after primary vaccination and 89% (39/44) had anti-HBs titers > 100 IU/ liter after primary vaccination. For the full term infants the percentages were 98% (816/829) and 93% (772/829) respectively. Neither for the limiting value of 10 UI/liter nor for for the limiting value of 100 IU/liter are there any significant differences between the

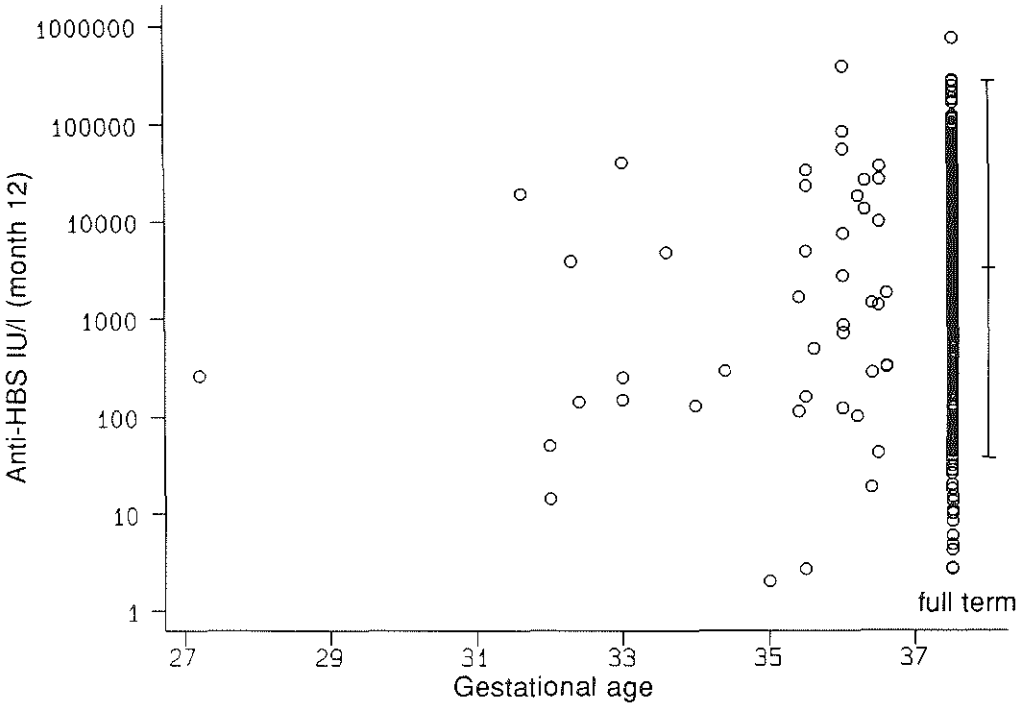

Figure 1. Titer anti-HBs of 44 preterm infants and of 829 full term infants after hepatitis B vaccination. Gestational ages of individually preterm infants (< 37.0 weeks) are shown on the horizontal axis; gestational ages of all full term infants (> 37.0 weeks) are shown together. (GMT= geometric mean titer; SD= standard deviation)

group of preterm infants and the group of full term infants. The percentage of children with anti-HBs titers >10 IU/liter from the total group (pre- and full- term) of infants vaccinated at birth (98%) was not significantly different from that from the total group (pre- and full- term) of infants not vaccinated until month 3 (99%). However, for the limiting value of 100 IU/liter (supposed to provide long lasting protection (12)) the difference between the group vaccinated at birth (90%) and the group vaccinated at month 3 (97%) proved significant ($p < 0,001$) (Table 2). Figure 1 shows the anti-HBs levels of the preterm and full term children at month 12, after completion of the primary vaccination series. Of 29 preterm infants anti-HBs titers taken 12 months after completion of the vaccination schedule (at month 24) were known: 97% of the preterm infants had anti-HBs titers > 10 IU/liter and 76% had anti-HBs titers > 100 IU/liter.

Nine preterm infants had a birth weight of less than 2000 grams; 6 of these had anti-HBs titers > 100 IU/liter after primary vaccination, 2 preterm infants with birthweights of 1880 and 1320 grams respectively, had anti-HBs titers between 10 and 100 IU/liter (both had a gestational age of 32.0 weeks and were vaccinated

Table 2. Preterm infants and full term infants vaccinated with hepatitis B vaccine from birth onwards or from 3 months onwards; number of infants with anti-HBs levels > 10 IU/L and > 100 IU/L 1-2 months after primary vaccinations, respectively.

start vaccination		titer anti-HBs >10 IU/L	titer anti-HBs >100 IU/L
from birth	preterm	95% (21/22)	86% (19/22)
onwards	full term	98% (431/439)	90% (394/439)
	total	98% (452/461)	90% (413/461)*
from 3 months	preterm	100% (22/22)	95% (21/22)
onwards	fullterm	99% (385/390)	97% (378/390)
	total	99% (407/412)	97% (399/412)*

* Fisher's exact test: $p < 0.001$

according to schedules 10 and 11 respectively). One infant, born pre- and dysmaturely (gestational age 35.0 weeks, birth weight 1955 grams), had an anti-HBs titer < 10 IU/liter after primary vaccination according to schedule 4. Of the 35 preterm infants with birth weights ≥ 2000 grams no child had an anti-HBs titer less than 10 IU/liter after completion of the vaccinations and 2 children had anti-HBs titers between 10 and 100 IU/liter. There was no significant difference in this respect between the group of preterm infants < 2000 grams and the group of preterm infants ≥ 2000 grams.

Four preterm infants had passively acquired anti-HBs (anti-HBs titers from umbilical cord blood ranging from 250 to 16250 IU/liter). With 3 of those infants the level of anti-HBs rose after primary vaccination and with one infant the level fell from 16250 to 1400 IU/liter after primary vaccination according to schedule 11. The 4 preterm infants with titers between 10 and 100 IU/liter were all vaccinated according to different schedules (schedules 1, 8, 10 and 11 respectively).

The one infant born pre- and dysmaturely (35.0 weeks, 1955 grams, several weeks of artificial respiration) whose response after primary vaccination was severely inadequate (anti-HBs titer 2 IU/liter) received a complete new series of vaccinations during its second year and then developed a response of over 3000 IU/liter anti-HBs. Eight full term low-responders (titers anti-HBs ≤ 10 IU/liter) similarly developed an adequate immune response after revaccination in the course of their second year. One of the 44 preterm infants eventually became an hepatitis B

carrier (HBsAg-positive) at the age of 5. Its anti-HBs titer after primary vaccination according to schedule 2 was 45 IU/liter, at month 12 (after the booster vaccination) it was only 6 IU/liter. Of the 827 full term infants 8 became HBsAg-positive.

The anti-HBs titers at month 3 of 2 preterm infants (gestational age 33.0 and 27.0 weeks, vaccinated according to schedules 2 and 3 respectively) who had received several exchange transfusions, were only 20 and 16 IU/liter respectively. In both cases the deviation from the geometric mean titer (GMT) is more than twice the standard deviation (SD) (GMTs for schedules 2 and 3 are 34 and 42 IU/liter respectively). At month 12 both children had anti-HBs titers > 100 IU/liter.

In neither group (preterm and full term infants) any serious side effects of the hepatitis B vaccine were reported.

Discussion

This study shows that 97% of preterm infants vaccinated with hepatitis B vaccine at chronolological age can develop an adequate immune response to hepatitis B without notable side effects. This percentage is similiar to that of full term infants with an adequate immune response after hepatitis B vaccination (1,2). The percentage of preterm infants with anti-HBs titers remaining > 10 IU/liter during their second year, was 97, which is even higher than the percentage found in the literature (94%) (12).

The immune response to hepatitis B vaccinatie depends from genetic as well as environmental factors (3-6). No serious diseases weakening the immune system were known to occur with either the preterm or the full term infants. No HLA-typing was done for either group, since there is no reason to assume the HLA-type corresponding with low immune responses to be specific for preterm infants. Anti-HBs titers taken after vaccination from children with passively acquired anti-HBs are difficult to interpret. Previous results, however, give no reason to suppose anti-HBs of maternal origin to impede an effective immune response (even after very low doses hepatitis B vaccine) (13). This assumption is supported by the rise, after primary vaccination, of the anti-HBs titers of 3 of the 4 preterm infants with positive anti-HBs.

Some people suggest that children weighing less than 2000 grams at birth react less well to hepatitis B vaccination than children with normal birth weight, and advise to postpone vaccination until normal weight it reached (14). In our study we found no significant difference in adequate immune response between infants < 2000 grams and infants ≥ 2000 grams. We therefore see no reason to postpone hepatitis B vaccination on the grounds of a too low birth weight. The ability to develop antibodies increases with age; however, Dancis et al. supposed

early exposition to antigens to be more important to the formation of antibodies than the stage of maturation of the immune system (15). Our study too showed preterm infants vaccinated at chronological age to be able at an early age to build an adequate immune response after hepatitis B vaccination. However, comparison of anti-HBs GMTs of both groups is difficult because of the extent of the range of anti-HBs titers of both preterm and full term infants in the various vaccination schedules (Figure 1), and also because of the relatively small number of preterm infants per vaccination schedule. What does appear to effect the anti-HBs titers is the starting point of the active immunization, irrespective of the gestational age. A starting point for active hepatitis B immunization at month 3 tends to lead to higher anti-HBs titers than a starting point at birth, which points to the importance of a longer exposition to antigens before the formation of antibodies.

The vaccination schedule as adopted in the Netherlands, which administers HBIg (CLB, 300 IU) to children born to hepatitis B surface antigen-positive mothers at birth and starts active immunization at month 3, must be considered superior to schedules starting vaccination at birth (16). The schedule is synchronic with the DTPP (diphteria, pertussis, tetanus, polio) vaccination schedule, which greatly enhances the practicability (7). Recently it has become known that preterm infants can develop an immune response to DTPP comparable with that of full term infants, although few data are available for very young (< 28 weeks) preterm infants (9-11). Our study too included only one infant with a gestational age of less than 28 weeks.

Our study included 2 children who received several exchange transfusions. Both children had relatively low anti-HBs titers from month 3 onwards. The HBIg administered passively at birth bridges the gap between birth and month 3, the starting point for active immunization. With an exchange transfusion the plasma of a child of an HBsAg-positive mother is replaced shortly after HBIg is administered, which might theoretically lead to inadequate protection (anti-HBs titer < 10 IU/liter) against hepatitis B, if active immunization has not yet started. This leads to an increased risk of hepatitis B infection. Administration of additional doses of HBIg to infants (both preterm and full term) receiving exchange transfusions might prevent this increase. High risk of infection through the mother (vertical transmission) makes such prevention particularly desirable. Indications of the risk of vertical transmission include signs of active viral replication (mother HBeAg- and HBV-DNA-positive as well as HBsAg-positive). Investigations into the effects of exchange transfusions on passively acquired anti-HBs titers would give us more insight into this hypothesis.

This study so far supports the recommendation of the Health Authorities to start hepatitis B vaccination of preterm infants at the appropriate chronological age, without correcting for their gestational age.

Acknowledgement

We thank Ir. W.C.J. Hop, statistician Erasmus University Rotterdam and Prof. Dr. P.J.J. Sauer for their critical comments on an earlier version of this manuscript. This study was supported by a grant from the Praeventiefonds, the Netherlands (no. 28-760-2).

References

1. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; 2: 1099-102.
2. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY et al. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984; 1: 921-6.
3. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321: 708-12.
4. Mazel JA. Preventie van perinatale hepatitis B bij pasgeborenen in Nederland. Proefschrift 1986.
5. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing hemodialyses. Acta Paediatr Scand 1985; 74: 213-8.
6. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H et al. Hepatitis B vaccination and immune response in children with malignant diseases. Eur J Pediatr 1985; 144: 160-3.
7. Geneeskundige Hoofdinspectie van de Volksgezondheid. Preventie hepatitis B bij pasgeborenen. GHI-bulletin 1989 september.
8. Verbrugge HP. Vaccinatie van praematuren. Tijdschr Kindergeneeskd 1986; 54: 28.
9. Bernbaum JC, Daft A, Anolik R, Samuelson J, Barkin R, Douglas S et al. Response of preterm infants to diphtheria-tetanus-pertussis immunizations. J Pediatr 1985; 107: 184-8.
10. Koblin BA, Townsend TR, Muñoz A, Onorato I, Wilson M, Polk F. Response of preterm infants to diphtheria-tetanus-pertussis vaccine. Pediatr Infect Dis J 1988; 7: 704-11.
11. Pullan CR, Hull D. Routine immunisation of preterm infants. Arch Dis Child 1989; 64: 1438-41.
12. Jilg W, Schmidt M, Deinhardt F. Persistence of specific antibodies after hepatitis B vaccination. J Hepatol 1988; 6: 201-7.
13. Moyes CD, Milne A, Dimitrakakis M, Goldwater PN, Pearce N. Very-low-dose hepatitis B vaccine in newborn infants: an economic option for control in endemic areas. Lancet 1987; 1: 29-31.
14. Genesca J, Esteban JJ, Esteban R. Hepatitis B immunoprophylaxis of low-birth-weight infants. Pediatr 1985; 76: 1020.
15. Dancis J, Osborn JJ, Kunz HW. Studies of the immunology of the newborn infant. Antibody formation in the premature infant. Pediatr 1953; 12: 151-7.
16. Schalm SW, Mazel JA, de Gast GC, Heijtink RA, Botman MJ, Bänffer JRJ et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatr 1989; 83: 1041-8.

Chapter 7

Hepatitis B revaccination of neonates with inadequate response after primovaccination

Canho del R, Schalm SW, Heijtink RA

Vaccine 1992; 10: 69

Introduction

Passive-active immunization with hepatitis B immunoglobuline and hepatitis B vaccine has proved to be highly effective in preventing perinatal transmission of hepatitis B infection (1-2). An anti-HBs antibody level > 10 IU/l is considered to provide protection against hepatitis B infection (3). Response to the vaccine depends on host factors like age, HLA type (4) and the presence of diseases affecting immunity (5-6). The immune response (expressed in IU/l) is higher in neonates than in adults (7). Still, inadequate responses to hepatitis B vaccine do occur. In adults additional vaccination in non- and low-responders has been reported to yield responses in 27-100% (8-10). The question: how to manage nonresponders, also arises for young children. As far as we know no data are available on additional vaccination in infants. Therefore, we report the results of revaccination offered to 9 healthy neonates who developed less or equal than 10 IU/l anti-HBs after primovaccination and a booster dose.

Methods

During the past 9 years 705 healthy newborns from HBsAg positive mothers received HBIg (120-300 IU, CLB, Amsterdam, The Netherlands) at birth and were vaccinated within the first year with plasma or recombinant-DNA vaccine according to several schemes including at least 2 initial doses and a booster dose.

Vaccine was always stored at 4 °C and given into the quadriceps muscle by physicians. HBsAg and anti-HBs were measured (Ausria II, Ausab, Abbott Laboratories) one month after completion of the immunization schedule. Nine infants with a negative test for HBsAg and an anti-HBs level ≤ 10 IU/l received 3-4 additional doses of plasma-derived (10 μg) or recombinant-DNA (20 μg) vaccine in their second year of life.

Results

After revaccination all infants showed an anti-HBs response above 10 IU/l; 7 children (78%) developed > 50 IU/l anti-HBs and four of them more than 100 IU/l. The three non-responders after primovaccination (anti-HBs 0 IU/L) had a response in the lower range (figure 1). No hepatitis B infections were observed among these nine children during follow-up (median 51, range 33-92 months). Twelve months after completion of revaccination 8 of the 9 infants (89%) still had more than 10 IU/l anti-HBs.

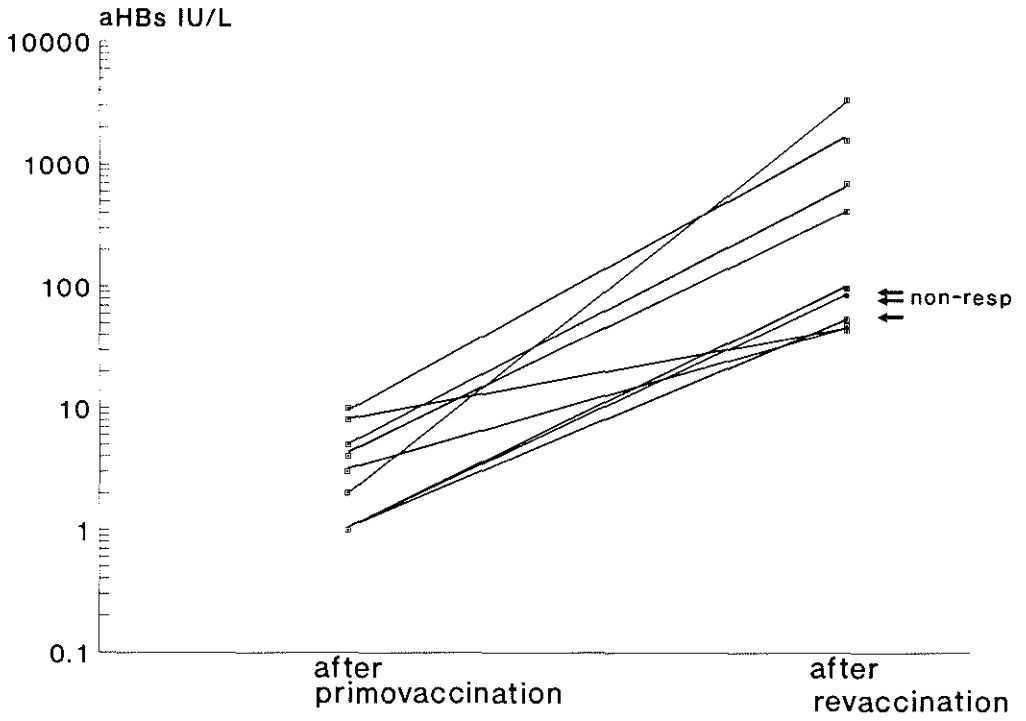

Figure 1. Anti-HBs response after hepatitis B revaccination in infants, who were low-responder (n=6) or non-responder (n=3) after primo vaccination and a booster dose. The non-responders are indicated by arrows.

Discussion

Our data show that additional vaccination of infants, who had a non-detectable or a weak response after primovaccination and no signs of hepatitis B infection, can yield responses with a high likelihood of protection against hepatitis B infection. From an epidemiological viewpoint this result is important since many of these children remain at risk for hepatitis B infection for years due to family contacts.

References

1. Beasley RP, Hwang LY, Lee GC, Lan C, Roan C, Huang F. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-102.
2. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987; 257: 2612-6.
3. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. Hepatology 1981; 1: 377-85.
4. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321: 708-12.
5. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing hemodialysis. Acta Paediatr Scand 1985; 74: 213-8.
6. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H et al. Hepatitis B vaccination and immune respons in children with malignant diseases.Eur J Pediatr 1985; 144: 160-3.
7. Jilg W, Schmidt M, Deinhardt F. Persistence of specific antibodies after hepatitis B vaccination. J Hepatol 1988; 6: 201-7.
8. Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986; 315: 209-14.
9. Jilg W, Schmidt M, Deinhardt F. Immune response to hepatitis B revaccination. J Med Virol 1988; 24: 377-84.
10. Wismans P, Hattum van J, Stelling T, Poel J, Gast de GC. Effect of supplementary vaccination in healthy non-responders to hepatitis B vaccination. Hepato-gastroenterol 1988; 35: 78-9.

Chapter 8

Human Leucocyte Antigens (HLA) in neonates with an inadequate response to hepatitis B vaccination

Canho del R, Vries de RRP, Schalm SW

Short version of this manuscript: Vaccine 1993; 11: 983

Introduction

Immunization with hepatitis B vaccine has proven to be highly effective in preventing transmission of hepatitis B infection (1-2). Nevertheless, inadequate responses to hepatitis B vaccine occur and vaccine recipients may therefore remain susceptible to hepatitis B virus infection. Factors that influence the response to vaccination include age (3) and the immunogenetic state of the vaccine recipient; the frequency of Human Leucocyte Antigens (HLA)-DR3 was increased in those who did not develop an adequate response to hepatitis B vaccination (4). Age appears to be an important factor in the mechanism of non/low responsiveness in adults (rate approximately 25%) but not in neonates (non/low response rate less than 10%) (5). To determine the importance of the immunogenetic factor for the outcome of hepatitis B vaccination in neonates, we investigated the HLA type of infants with an inadequate response to hepatitis B vaccination.

Methods

Between 1982 and 1991, 705 healthy newborns from HBsAg-positive mothers received HBIg (150-300 IU, CLB, Amsterdam, The Netherlands) at birth and were vaccinated within the first year with plasma or recombinant-DNA vaccine* according to a three or four-dose vaccination schedule. Anti-HBs and HBsAg were measured at 3, 6, 11 and 12 months of age. Infants who did not respond adequately to the vaccination (anti-HBs < 10 IU/L at month 12) and were still HBsAg-negative were revaccinated in their second year of life. Sixteen of the 705 newborns (2.3 %) had anti-HBs titres < 10 IU/L. In December 1991, 8 infants (7 Caucasians and 1 from Cape Verde Islands) with an anti-HBs level below 10 IU/L and a negative test for HBsAg (noninfected low responders) and another 8 infants (6 Caucasians, 2 Asians) who were anti-HBs negative but HBsAg- positive (infected nonresponders) were identified.

HLA typing for class I and II antigens was performed, using microcytotoxity test on peripheral blood mononuclear cells (Tissue Typing Laboratory, head: Dr. G.M.Th. Schreuder, Department of Immunohaematology and Blood bank, University Hospital, Leiden, The Netherlands). Anti-HBs and HBsAg levels were assessed with commercial radioimmunoassays (Ausria II, Ausab, Abbott Laboratories Chicago, ILL, USA).

*10 μg plasma vaccine and 5 μg and 10 μg recombinant vaccine (Merck, Sharp and Dohme, Westpoint Pa, United States of America). 20 μg recombinant vaccine (SmithKline Biologicals, Rixensart, Belgium).

Results

Table 1 shows the HLA phenotypes of the 8 noninfected low responders and the 8 infected nonresponders to hepatitis B vaccine. HLA-DR3 was present in 4 of the 8 (50%) noninfected low responders and none of the 8 (0%) infected nonresponders. Two noninfected low responders (numbers 1 and 2) were probably homozygous for HLA-DR3. None of the infants were homozygous for the HLA-B8-DR3 haplotype. A rough estimate of expected DR3 homozygotes in this ethnic group is 1-2%.

Discussion

This study suggests that the HLA-DR3 haplotype plays a role in the low responsiveness to hepatitis B vaccination in noninfected neonates. Response to the vaccine depends on host factors such as age (3), the presence of diseases affecting immunity (6-9) and HLA type (4,10-15). There is evidence that Caucasian individuals homozygous for HLA-B8,SC01,DR3 lack an immune response gene for HBsAg and produce much lower levels of antibody against hepatitis B vaccine than individuals heterozygous for or lacking this haplotype (10,15). Moreover the frequency of HLA-DR3 (30%) was significantly increased in individuals who did not develop an adequate response to hepatitis B vaccination in the absence of HBsAg (noninfected lowresponders) (4,13). In our study with only a small number of ethnically heterozygous individuals, it is striking that all 4 DR3 positive children were in the group of noninfected low responders and that two of them were probably homozygous for DR3. These low responders were not absolute low responders, since all of them developed protective anti-HBs levels after hepatitis B revaccination in their second year of life, as described recently (16). The 2 children homozygous for HLA-DR3 produced anti-HBs in the lower range (45 en 55 IU/L) after revaccination in comparison to the other 6 revaccinated low responders (median 171, range 49-3497 IU/L), which is consistent with the hypothesis of a recessive-DR3 associated low responsiveness to hepatitis B vaccine (10,15). The observation that none of the 8 nonresponders, who became infected with hepatitis B virus, was DR3 positive suggests that HLA-associated low responsiveness is not causally related to this type of failure of hepatitis B vaccination.

Table 1. HLA type for 8 noninfected low responders and 8 infected nonresponders to hepatitis B vaccine.

group		HLA type									
noninfected low responders	ethnics*	A		B		C		DR		DQ	
1	Medit.	2	3	7	**8**	7	-	**3**	-	2	-
2	Neth.	3	30	62	18	3	5	**3**	-	2	-
3	Medit.	3	30	13	35	4	6	1	**3**	2	5
4	Neth.	1	11	**8**	56	1	7	1	**3**	2	5
5	Medit.	26	11	18	35	4	7	4	-	7	8
6	Medit.	2	11	35	53	4	4	-	11	7	8
7	Cap. V.	2	23	58	-	2	-	13	11	6	7
8	Medit.	3	28	60	72	10	-	13	15	6	-
infected nonresponders											
1**	Medit.	11	28	18	48	7	-	4	14	5	7
2**	Medit.	11	28	18	48	7	-	4	14	5	7
3	Medit.	2	24	49	35	4	-	7	-	2	-
4	Medit.	3	-	35	37	4	-	11	-	7	-
5	Medit.	3	24	35	-	4	-	11	8	7	4
6	Asia	3	33	58	-	10	-	15	13	6	1
7	Medit.	1	33	52	63	10	4	15	7	6	2
8	Asia	24	11	55	61	1	10	11	13	6	7

* Ethnic background: Mediterranean, the Netherlands, Cape Verde Islands, Asia.
** Infected nonresponders 1 and 2 are brother and sister.
- Possible homozygous

References

1. Beasley RP, Hwang LY, Lee GC, Lan C, Roan C, Huang F. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-102.
2. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. Jama 1987; 257: 2612-6.
3. Hess G, Hingst V, Cseke J, Boc HL, Clemens R. Influence of vaccination schedules and host factors on antibody response following hepatitis B vaccination. Eur J Clin Microbiol Infect Dis 1992; 11: 334-40.
4. Varla-Leftherioti M, Papanicolaou M, Spyropoulou M, Vallindra H, Tsiroyianni P, Tassopoulos N et al. HLA associated non responsiveness to hepatitis B vaccine. Tissue Antigens 1990; 35: 60-3.
5. Lok ASF, Lai CL, Wu PC, NG MMT. Response to hepatitis B vaccine in family members of HBsAg carriers. J Med Virol 1986; 19: 33-9.
6. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing hemodialysis. Acta Paediatr Scand 1985; 74: 213-8.
7. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H et al. Hepatitis B vaccination and immune response in children with malignant diseases. Eur J Pediatr 1985; 144: 160-3.
8. Carne CA, Weller IVD, Waite J, Briggs M, Pearce F, Adler MW, Tedder RS. Impaired responsiveness of homosexual men with HIV antibodies to plasma dereived hepatitis B vaccine. Br Med J 1987; 294: 866-8.
9. Heijtink RA, Jong de P, Schalm SW, Masurel N. Hepatitis B vaccination in Down's syndrome and other mentally retarded patients. Hepatology 1984; 4: 611-4.
10. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321: 708-12.
11. Watanabe H, Matsushita S, Kamikawaji N, Hirayama K, Okumura M, Sasazuki T. Immune suppression gene on HLA-Bw54-DR4-DRw53 haplotype controls nonresponsiveness in humans to hepatitis B surface antigen via CD8+ suppressor T cells. Hum Immunol 1988; 22: 9-17.
12 Marescot MR, Budkowska A, Pillot J, Debre P. HLA linked immune response to S and pre-S2 gene products in hepatitis B vaccination. Tissue Antigens 1989; 33: 495-500.
13. Craven DE, Awdeh ZL, Kunches LM, Yunis EJ, Dienstag JL, Werner BG et al. Nonresponsiveness to hepatitis B vaccine in health care workers. Results of revaccination and genetic typings. Ann Int Med 1986; 105: 356-60.
14. Pol S, Legedre C, Mattlinger B, Berthelot P, Kreis H. Genetic basis of nonresponse to hepatitis B vaccine in hemodialyzed patients. J Hepatol 1990; 11: 385-7.
15. Kruskall MS, Alper CA, Awdeh Z, Yunis EJ, Marcus-Bagley D. The immune response to hepatitis B vaccine in humans: inheritance patterns in families. J Exp Med 1992; 175: 495-502.
16. Canho del R, Schalm SW, Heijtink RA. Hepatitis B revaccination of neonates with inadequate response after primovaccination. Vaccine 1992; 10: 69.

Chapter 9

Failure of neonatal hepatitis B vaccination: the role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates

Canho del R, Grosheide PM, Schalm SW, Vries de RRP, Heijtink RA

Journal of Hepatology: in press

Abstract

In a hepatitis B vaccination programme (1982-1992), 705 infants from HBsAg- positive mothers received HBIg within 2 hours of birth and were vaccinated according to a three- or four-dose vaccination schedule starting either at month 3 or directly after birth. Eight children became HBsAg-positive during the first year of life (group 1: infected nonresponders). In order to determine whether failure of the hepatitis B vaccination was due to perinatal maternal high-level viraemia or genetically determined infant nonresponsiveness to the vaccine, we measured HBsAg and anti-HBs levels in infants and HBeAg and HBV-DNA levels in maternal serum and determined the HLA type of the infants. Controls included 14 infants with a normal anti-HBs response 1 year after vaccination (group 2: non-infected responders) and all 8 infants without HBsAg and antiHBs 1 year after vaccination (group 3: noninfected low responders).

HBsAg, HBeAg and anti-HBs were measured by radioimmunoassay (Abbott Laboratories), HBV-DNA quantitatively by solution hybridization for groups 1, 2 and 3 (Abbott HBV-DNA assay, Abbott Laboratories); HLA was characterized by microcytotoxicity test for groups 1 and 3.

All infants in groups 1 and 2 were born of HBeAg carrier mothers, those in group 3 of HBeAg-negative mothers. HBV-DNA levels in maternal serum in group 1 were significantly higher than in group 2 (Wilcoxon rank-sum test: $p < 0.01$). HBV-DNA was not observed in group 3 maternal serum samples. HLA B8 and DR3 were not found in group 1 but were present in 4/8 and 2/8 infants of group 3, respectively.

Failure of current passive-active hepatitis B immunization appears to be related not to genetic nonresponsiveness of infants but rather to perinatal maternal high-level viraemia. HBV-DNA assay of HBeAg-positive mothers may identify those infants in need of additional action to lower the risk of vertically transmitted HBV infection.

Introduction

Passive-active immunization with hepatitis B immune globulin (HBIg) and hepatitis B vaccine has proved to be highly effective in preventing perinatal transmission of hepatitis B virus (HBV) infection (1-2). Nevertheless, inadequate responses to hepatitis B vaccine occur; 1-2 % of properly vaccinated infants of HBsAg-positive carrier mothers become HBsAg-positive and another 1-2 % of the infants are not able to develop sufficient amounts of anti-HBs for longlasting immunity against HBV. An inadequate response is defined as an anti-HBs titre of less than 10 IU/L after completion of the immunization schedule in infants with HBsAg or without HBsAg positivity. Failure of immunoprophylaxis could be due to either in utero infection (3); a high dose of HBV, transmitted during delivery and related to maternal high-level viraemia (4-7); insufficient neutralization capacity of the HBIg injection; or failure to respond adequately to the vaccine, given in an adequate dose, due to genetic (8-11) or acquired causes (12-15). We analysed some of the possible causes of hepatitis B prophylaxis failure in neonates of HBsAg carrier mothers in order to determine the need for modified action for those babies at high risk for vertically transmitted HBV infection.

Methods

Between 1982 and 1991, 705 healthy newborns of HBsAg-positive mothers received 0.5 ml HBIg/kg bodyweight (100 IU anti-HBs/ml, CLB, Amsterdam, The Netherlands) at birth and were vaccinated within the first year with plasma or recombinant-DNA vaccine* according to a three- or four-dose vaccination schedule (16).

Eight children became HBsAg-positive during the first year of life (**group 1: infected nonresponders**). In order to determine whether the causes of the failure of hepatitis B vaccination were due to perinatal maternal high-level viraemia in combination with pregnancy-associated maternal-foetal transfusion or genetically determined infant nonresponsiveness to the vaccine, we measured HBsAg and anti-HBs levels in infants and HBeAg and HBV-DNA levels in maternal serum taken during pregnancy or just before delivery and determined the HLA type of the infants. As controls, we included 14 HBsAg-negative infants with a normal anti-HBs (> 100

*10 μg plasma vaccine and 5 μg and 10 μg recombinant vaccine (Merck, Sharp and Dohme, Westpoint Pa, United States of America). 20 μg recombinant vaccine (SmithKline Biologicals, Rixensart, Belgium).

Table 1. Anti-HBs level and onset of HBsAg-positivity in 8 infected nonresponders (group 1) to hepatitis B vaccine.

infected nonresponders	anti-HBs (IU/L) 3	6	11	12	months	HBsAg 3	6	11	12
1	0	0	0	0		+	+	+	+
2	0	0	0	0		+	+	+	+
3	0	3	5	28†		+	+	+	+
4	16	2	0	0		-	+	+	+
5	29	0	0	6†		-	+	+	+
6	58	0	*	0		-	*	+	+
7	38	17	0	0		-	-	+	+
8	45	39	0	0		-	-	+	+

*no test result available
† heterotypical anti-HBs

Table 2. HLA-B8-DR3 type in 16 non/low responders to hepatitis B vaccine.

		HLA type			
	total	B8+DR3-	B8-DR3+	B8+DR3+	B8-DR-
infected nonresponders	8	0	0	0	8
noninfected low responders	8	0	2*	2*	4

*1 was homozygous for DR3 antigen

Risk factors for maternal-foetal transfusion

Risk factors for pregnancy-associated maternal-foetal transfusion, including threatened abortion, amniocentesis, chorion villus sampling and assisted delivery (vacuum, forceps), were not present.

IU/L) response to vaccination (**group 2: noninfected responders**) and all 8 HBsAg-negative infants with an anti-HBs level of less than 10 IU/L one month after completion of the immunization schedule (**group 3: noninfected low responders**). The fourteen noninfected responders, chosen at random, were matched with the infected nonresponders for maternal HBeAg and the vaccination schedule used.

HBsAg, HBeAg and anti-HBs were assessed using commercial radioimmunoassays (Abbott Laboratories,Chicago, Ill, USA). HBV-DNA was quantitated by solution hybridization for groups 1, 2 and 3 (HBV-DNA assay, Abbott Laboratories, USA). HLA typing for class I and II antigens was performed for groups 1 and 3 using a microcytotoxicity test on peripheral blood mononuclear cells (Tissue Typing Laboratory, Department of Immunohaematology and Blood bank, University Hospital, Leiden, The Netherlands).

In order to exclude HBV variants, escaping anti-HBs control, serum samples of HBsAg infants, taken between 1 and 7 year of age, were diluted to about 3 ng/ml and incubated for 2 hours at room temperature with various amounts of HBIg (1-4094 IU/L). Residual HBsAg was measured thereafter in the HBsAg assay (neutralization in solution assay). Full neutralization is defined as reduction of HBsAg to the level of the negative control sample in the HBsAg assay.

Statistical comparison of HBV-DNA levels was performed by means of the Wilcoxon rank-sum test.

Results

Onset of HBsAg-positivity and anti-HBs levels in group 1 (table 1)

Three infants of group 1 were HBsAg-positive from 3 months of age onwards without detectable anti-HBs. In one of these 3 children, presumably vaccine-induced heterotypical anti-HBs increased slowly during the first year of follow up. Five infants developed HBsAg between 3-11 months after birth. Low anti-HBs levels (< 50 IU/L), probably still derived from HBIg, were measured in the preceding months.

HBsAg neutralization in group 1

In an in vitro assay, full neutralization of HBsAg of HBsAg-positive infants was obtained in all cases with 1000-4000 IU/L HBIg (neutralization in solution assay), indicating that the HBV infection in the infants is not the result of mismatching between HBIg and variant HBsAg.

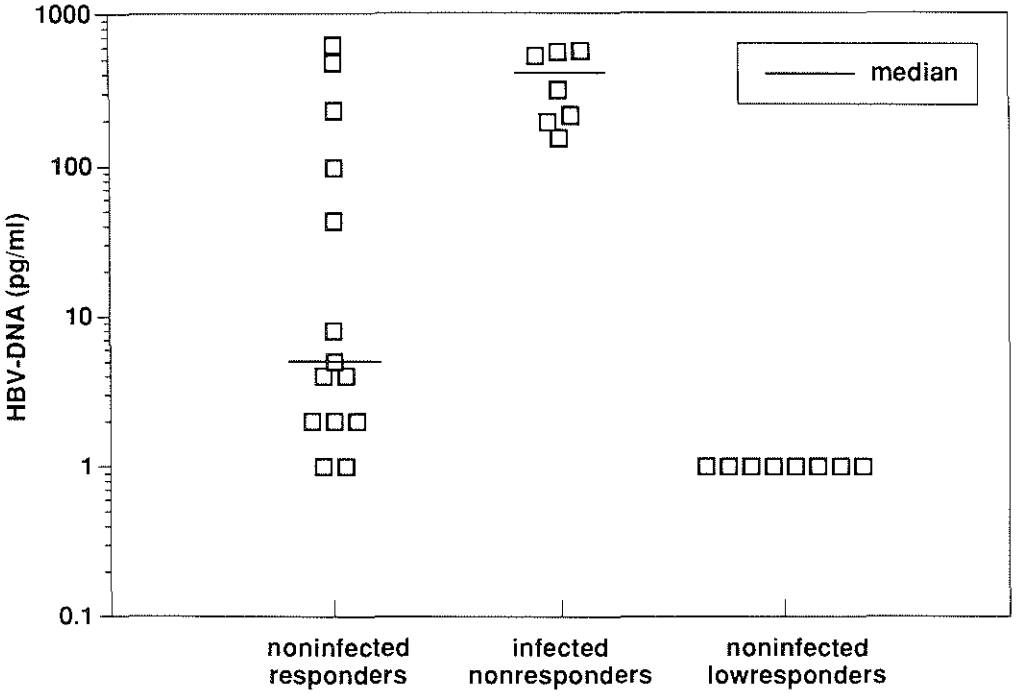

Figure 1. HBV-DNA levels in HBeAg-positive mothers of 7 infected nonresponders (median 314, range 152-567 pg/ml) and 14 noninfected responders (median 4.5, range 0-618 pg/ml) and in 8 HBeAg-negative mothers of noninfected responders to hepatitis B vaccine (all negative).*

* maternal HBV-DNA of 1 infected nonresponder is not available

Maternal level viraemia

HBeAg

118/705 (16.7%) HBsAg positive mothers were also positive for HBeAg. All 8 infected nonresponders (group 1) and 14 noninfected responders (group 2) were born of HBeAg carrier mothers. Eight noninfected low responders (group 3) were born of HBeAg-negative mothers.

HBV-DNA

Figure 1 shows the HBV-DNA levels in maternal serum for group 1, group 2 and group 3. The HBV-DNA level in maternal serum was significantly higher in group 1 than in group 2 (Wilcoxon rank-sum test, $p < 0.01$). All 8 maternal HBV-DNA assays in group 3 (HBeAg negative mothers) were negative.

HLA type in infants

HLA-B8-DR3 was not found in any infants in group 1 whereas 4 infants in group 3 were HLA-DR3 positive and of which 2 were also HLA-B8 positive. Table 2 shows the HLA characteristics of groups 1 and 3. Two noninfected low responders were probably homozygous for HLA-DR3 (a rough estimate of expected DR3 homozygotes in this ethnic group is 1-2%).

Discussion

The present study shows that in this series failure of hepatitis B vaccination was most likely due to high perinatal levels of HBV-DNA in the HBeAg carrier mothers. We found no evidence for genetically determined nonresponsiveness to the vaccine in HBV-infected children. It is believed that intra-uterine HBV infection occurs in 1-2% (3/235) of infants of HBeAg positive HBV carrier mothers (3). For a similar incidence (1-2%) in our study, intra-uterine infections can be expected in 2/118 infants of HBeAg carrier mothers. Eight HBsAg positive infants were detected in our study, that means that there are remaining causes of failure of hepatitis B vaccination. The HBV-DNA levels in HBsAg, HBeAg carrier mothers whose babies became infected were significantly higher than those in corresponding mothers whose babies were protected by passive and active immunization, which is consistent with previously published results (4-7). These HBV-DNA levels were much higher than the risk level of 5 pg/ml, reported by Ip and colleagues (5). We also found a lower frequency of the HBV carrier state for infants (6.8% (8/118) of infants at risk for HBV). Ip et al. detected 12.7% (14/110) HBsAg positive infants from infants at risk for HBV, despite passive-active hepatitis B immunization (5). An explanation may be the higher doses of both HBIg and vaccine used in our study. The amount of HBIg was probably not even high enough in these infants, because anti-HBs levels dropped down after 3 months of age or even earlier and HBsAg became or remained positive. Until now we did not observe a-deficient HBV mutants in The Netherlands, as seen in Italy (17). The fact that we did not see a coexistence between HBsAg and anti-HBs in 7 infants and that full neutralization of HBsAg was obtained by HBIg in all 8 infants assumes no surface antigen variants in our study population. Apart from in utero infection, HBV infection in infants born to HBeAg carriers with high serum levels of HBV-DNA can possibly be avoided by additional doses of HBIg given at birth - to increase the capacity to neutralize HBV - followed by active immunization, or by caesarean section - to reduce the amount of HBV acquired by maternal-foetal transfusion- together with passive and active immunization at birth (18,19).

Assaying HBV-DNA of HBeAg positive mothers (in areas with a low incidence of the precore mutant), may identify those infants in need of additional action to lower the risk of vertically transmitted HBV infection.

Acknowledgement

The authors thank all participants of the Dutch study group Prevention Neonatal Hepatitis B: Wladimiroff JW, Dept. of Obstetics, Academic Hospital Dijkzigt, Rotterdam; Nuijten ASM, State School of Training for Obstetricians, Rotterdam; Zwijnenberg J, Dept. of Pediatrics, Ruwaard van Putten Hospital, Rotterdam; Bänffer JRJ, Regional Public Health Laboratory, Rotterdam; Mazel JA, General Practice, Borne; Botman MJ, Regional Laboratory of Pathology and Microbiology, Enschede; Gerards LJ, University Hospital Utrecht, Wilhelmina Children's Hospital, Utrecht; Gast de GC, Blood Transfusion Laboratory, Utrecht; Christiaens GCML, Dept. of Gynaecology and Obstetrics, Academic Hospital, Utrecht; Fetter WPF, Dept. of Pediatrics, St. Sophia Hospital, Zwolle; Baerts W, Dept. of Pediatrics, St. Sophia Hospital, Zwolle and Dr. G.M.Th. Schreuder, head of Tissue Typing Laboratory, Dept. of Immunohaematology and Blood bank, University Hospital Leiden, the Netherlands (for HLA typing).

This study was supported by a grant from the Praeventiefonds, the Netherlands (no. 28-760-2).

References

1. Beasley RP, Hwang LY, Lee GC, Lan C, Roan C, Huang F et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-102.
2. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. Jama 1987; 257: 2612-6.
3. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY et al. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984; i: 921-6.
4. Lin HH, Chang MH, Chen DS, Sung JL, Hong KH, Young YC et al. Early predictor of the efficacy of immunoprophylaxis against perinatal hepatitis B transmission: analysis of prophylaxis failure. Vaccine 1991; 9: 457-60.
5. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW . Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989; i: 406-10.
6. Boxall EH, Harrison TJ, Wheeley SM. HBV-DNA levels in hepatitis B carrier mothers: relationship with protection against perinatal transmission by vaccine. In "Viral Hepatitis and Liver Disease"; Proceedings of the 1990 International Symposium on viral hepatitis and liver disease. Williams and Wilkens 1991: 757-9.
7. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP et al. Prevention of maternal-infant hepatitis B virus transmission by immunization: The role of serum hepatitis B virus DNA. Hepatol 1986; 6: 369-73.
8. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321: 708-12.
9. Varla-Leftherioti M, Papanicolaou M, Spyropoulou M, Vallindra H, Tsiroyianni P, Tassopoulos N et al. HLA associated non responsiveness to hepatitis B vaccine. Tissue Antigens 1990; 35: 60-3.
10. Watanabe H, Matsushita S, Kamikawaji N, Hirayama K, Okumura M, Sasazuki T. Immune suppression gene on HLA-Bw54-DR4-DRw53 haplotype controls nonresponsiveness in humans to hepatitis B surface antigen via CD8+ suppressor T cells. Hum Immunol 1988; 22: 9-17.
11. Kruskall MS, Alper CA, Awdeh Z, Yunis EJ, Marcus-Bagley D. The immune response to hepatitis B vaccine in humans: inheritance patterns in families. J Exp Med 1992; 175: 495-502.
12. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing hemodialysis. Acta Paediatr Scand 1985; 74: 213-8.
13. Carne CA, Weller IVD, Waite J, Briggs M, Pearce F, Adler MW, Tedder RS. Impaired responsiveness of homosexual men with HIV antibodies to plasma dereived hepatitis B vaccine. Br Med J 1987; 294: 866-8.
14. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H et al. Hepatitis B vaccination and immune response in children with malignant diseases. Eur J Pediatr 1985; 144: 160-3.
15. Heijtink RA, Jong de P, Schalm SW, Masurel N. Hepatitis B vaccination in Down's syndrome and other mentally retarded patients. Hepatology 1984; 4: 611-4.
16. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989; 83: 1041-7.
17. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E et al. Vaccine-induced escape mutant of hepatitis B vaccine. Lancet 1990; 336: 325-9.
18. Lee SD, Lo KJ, Tsai YT, Wu JC, Wu TC, Yang ZL et al. Role of Caesarean section in prevention of mother-infant transmission of hepatitis B virus. Lancet 1988; ii: 833-4.
19. Schalm SW, Pit-Grosheide P. Prevention of hepatitis B transmission at birth. Lancet 1989; i: 44.

Chapter 10

Follow up of hepatitis B infection in infants who became HBsAg positive in spite of hepatitis B immunization

Canho del R, Grosheide PM, Heijtink RA, Schalm SW and the Dutch study group Prevention Neonatal Hepatitis B

Dutch version of this manuscript has been accepted for publication in Nederlands Tijdschrift voor Geneeskunde

Abstract

Objective: To describe virological, clinical and biochemical characteristics of infants who became hepatitis B infected in spite of passive-active hepatitis B immunisation.

Patients: As part of the research programme of the study group "Prevention Neonatal Hepatitis B", 705 newborns of HBsAg positive mothers received hepatitis B immunoglobulines directly after birth, in the period 1982-1989. Active immunization with plasma vaccine or recombinant-DNA vaccine occurred in the first year of life according a three-dose or a four-dose schedule. Despite passive-active immunization, 9 children became positive for HBsAg.

Results: Median follow up was 5 years (range 3-8 years). Eight of the 9 infants were also positive for HBeAg. At the end of the follow up 1 child lost HBsAg and 2 children lost HBeAg. Only 1 child experienced a symptomatic hepatitis B infection with raised transaminase levels. The other 8 infants with chronic hepatitis B were not symptomatic and were in excellent health. Transaminase levels were ever normal in 7 infants.

Conclusion: Most of the infants who became HBsAg positive, in spite of passive-active hepatitis B immunisation, developed a chronic hepatitis B infection, without clinical and biochemical dysfunctions. On the basis of these findings and recent literature concerning possible therapy, guidelines are given for the follow up of children with chronic hepatitis B.

Introduction

The principal objective of hepatitis B vaccination programmes is the prevention of chronic hepatitis, cirrhosis, and hepato-cellular carcinoma. Passive-active immunization with hepatitis B immunoglobulin (HBIg) and hepatitis B vaccine has proven very effective (85-97%) in the prevention of perinatal transmission of hepatitis B infection (1-3).

This kind of infection chiefly occurs around the time of birth and without immuno-prophylaxis it leads to, usually prolonged (5), chronic hepatitis B carriership in 70-90% (4). In a Taiwanese study (5) only 9 out of 370 children (2/244 HBsAg-positive mothers, 7/126 HBsAg-negative mothers) lost HBsAg within 4.3 years (HBsAg loss < 1% per year). The incidence of the transition from active replication of the virus to virus latency (HBeAg sero-conversion) is also low: < 2% per year (6).

This article describes the virological, clinical, and biochemical aspects of the course of the hepatitis B infection of 9 children of HBsAg-positive mothers, who became hepatitis B carriers in spite of passive-active vaccination. These findings are compared with data from the literature on hepatitis B-positive children who were not vaccinated. On the basis of our findings and of recent literature on possible therapies, we offer guidelines for the treatment of young HBsAg-positve children.

Patients and methods

In the scope of a study by the "Prevention Neonatal Hepatitis B" group 705 neonates born to HBsAg-positive mothers (country of origin: The Netherlands n=83, Mediterranean n=74, Surinam n=74, Asia n=102, other n=69) received HBIg (150-300 IU, CLB, Amsterdam) at birth, during the period 1982-1889. Active vaccination with 10 μg plasma vaccine and 5 μg and 10 μg recombinant vaccine (Merck, Sharp and Dohme, Westpoint, USA) and 20 μg recombinant vaccine (SmithKline Beecham Biologicals, Rixensart, Belgium) was executed according to a 3-dose or a 4-dose schedule (7).

Blood sampling took place at birth and at months 3, 6, 11, and 12; thereafter annually during a median period of 5 years (range 2 to 8 years). All blood samples from the first year of life were tested for anti-HBs, anti-HBc, and HBsAg. HBsAg-negative children were subsequently tested annually for anti-HBs and anti-HBc.

Anamnesis, physical examination, HBsAg, anti-HBs, HBeAg and anti-HBc tests of the HBsAg-positive children were, if possible, carried out annually, and in addition their hepatic functions (ASAT, ALAT, Alk. phosphatase, alpha-feto-protein) were tested and an occasional echo abdomen was made. Serum was tested

once for antibodies against hepatitis delta (anti-HD), during the year that HBsAg-positivity was diagnosed.

The mother's blood was tested for HBeAg during pregnancy of just before delivery, and when found positive it was also tested for HBV-DNA.

HBsAg, anti-HBs, HBeAg, and anti-HBc were analyzed in the serum by means of radio-immuno-tests. Anti-HD was determined by an enzym-immuno-test. HBV-DNA was determined quantitively by means of a fluid hybridisation method (Abbott Laboratories, Chicago, Ill, USA).

Statistics

Clearance rates of HBsAg and HBeAg were computed by dividing the number of children that had become negative for HBsAg and HBeAg respectively, by the number of years of HBsAg-positivity observation and HBeAg-positivity observation respectively. 95% Confidence Intervals were computed for the clearance rates (8).

Results

Nine from the 705 children (6 boys and 3 girls) from our vaccination program became HBsAg positive. These 9 children were observed for a period of 3 to 8 years (median = 5 years).

Virology children

HBsAg

Onset of detection: 8 children became HBsAg-positive during their first year of life and 1 child at age 5. Of the 8 children 3 were already HBsAg-positive at month 3, the other 5 children became HBsAg-positive between month 3 and month 11 (Figure 1). The child that became HBsAg-positive at age 5 may have become infected through an HBeAg-positive brother.

Clearance rate: at the end of the follow-up 1 child had lost HBsAg at year 2 (nr. 6). Total clearance rate of HBsAg was 3% (95% CI: 0.4-22%) per year.

HBeAg

Onset of detection: 8 of the 9 children when found HBsAg-positive were HBeAg-positive as well; 1 child was HBeAg-negative (Figure 1).

Clearance rate: at the end of the follow-up 2 of these 8 HBeAg-positive children had become negative at year 2 and year 3 respectively. Total clearance rate of the 8 HBeAg- and HBsAg-positive children was 7% (95% CI: 3-19%) per year.

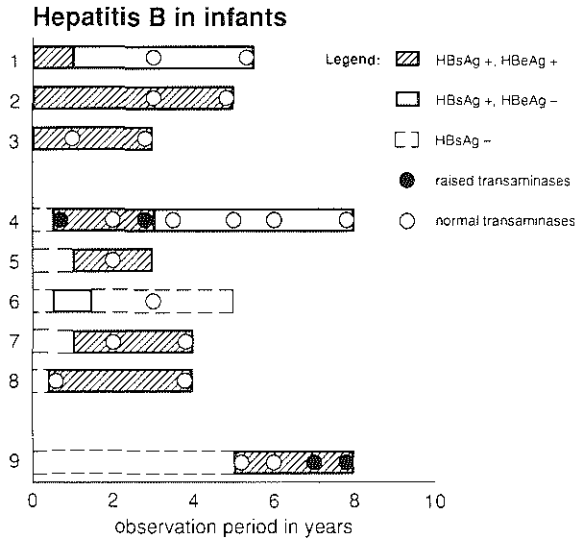

Figure 1. *Chronic hepatitis B infection in 9 infants despite hepatitis B vaccination.*

Table 1. *Anti-HBs level and onset of HBsAg positivity in eight infants who became HBsAg carrier despite passive-active immunisation in their first year of life.*

hepatitis B carriers	anti-HBs (IU/L) 3	6	11	12	months	HBsAg 3	6	11	12
1*	0	0	0	0		+	+	+	+
2*	0	0	0	0		+	+	+	+
3*	0	3	5	28†		+	+	+	+††
4	16	2	0	0		-	+	+	+
5	45	39	0	0		-	-	+	+
6	29	0	0	6†		-	+	+	+††
7	38	17	0	0		-	-	+	+
8	58	0	**	0		-	**	+	+

* intrauterine infection
** no test result available
† heterotypical anti-HBs
†† no signs for mutant-virus

Anti-HBs

In 2 of the 3 children that were HBsAg-positive from month 3 onwards, anti-HBs was never found in 2 children; 1 child developed anti-HBs, heterotypical with respect to the circulating HBsAg (Table 1). In the remaining 5 children that became HBsAg positive during their first year of life, anti-HBs of 16-58 IU/liter originating from hepatitis B immunoglobulin (HBIg) was found at month 3. After month 6 no anti-HBs or only heterotypical anti-HBs was detectable (Table 1). The HBsAg-positive child that lost HBsAg again in year 2 (nr. 6) developed anti-HBs from its second year, with titers varying from 10 to 50 IU/liter during 5 years of follow-up. The child that became HBsAg-positive at year 5 (Figure 1, nr. 9) showed a weak immune response after vaccination: anti-HBs titer of 43 IU/liter at month 12, 0 IU/liter at months 24-48.

Anti-HBc

Seven of the 9 children had anti-HBc from birth. Two children from 1 family were anti-HBc-negative until month 12; one of these developed anti-HBc between months 12 and 24, the other remains anti-HBc-negative until today (observation period 5 years). There is no reason to suppose a general immune dysfunction: both children are in excellent health and produce IgG-antibodies against Rubella and Measles vaccine. There are no indications for a mutated hepatitis B virus in these children (sequential analysis HBV-DNA, H. Will, Heinrich Pette Institut für Experimentele Virologie und Immunologie, Universität Hamburg, Germany; personal communication).

Anti-HD

Antibodies against hepatitis delta was found in none of the HBsAg-positve children during the first year after HBsAg-positivity was detected.

Virology mothers

HBeAg of 8 of the 9 mothers was positive. The child that became HBsAg-positive at year 5 had a HBeAg-negative mother. HBV-DNA of 7 HBeAg-positive mothers was median 314 pg/ml, range 152-567 pg/ml.

Clinical and biochemical data

Summer 1992, all children are in good health and attend primary school.
One child (nr. 4) suffered a symptomatic hepatitis B infection at month 7: insufficient feeding and groaning were reason for hospitalization. There was no icterus; serum transaminases were raised, ASAT 80-150 IU/liter, ALAT 200-300 IU/liter

(normal for neonates less than 80 IU/liter). Ultra-sound scanning showed an enlarged liver and speen, compatible with a diffuse process in the liver. Within a few months hepatic functions returned to normal, but HBsAg remained positive. The remaining 8 children were asymptomatic at the time of detection of HBsAg-positivity and showed no signs of hepatomegaly, splenomegaly, spider naevi or other characteristics of chronic hepatic disease. Serum transaminases, alpha-fetoprotein and ultra-sound scans of the liver were always normal. However, because of incomplete observations (only 4 children had ulta-sound scans and of only 3 children alpha-fetoprotein was determined) we cannot be certain about this. The child that became HBsAg-positive at year 5, developed abnormal transaminases (ALAT 55 U/liter, ASAT 54 U/liter; normal less than 30 IU/liter) at year 8.

Discussion

In our neonatal hepatitis B vaccination program 9 out of 705 children became HBsAg-positive. Only one child suffered an symptomatic accute hapatitis B infection, which resulted in chronic hepatitis B. During follow-up 8 of the 9 (89%) HBsAg-positive children remained HBsAg positive, without clinical abnormalities and 6 of these also without biochemical abnormalities. These data are consistent with other findings that the majority of children is asymptomatic at the time of onset of HBsAg-positivity (9) and that 80-90% of HBsAg-positive infants develops chronic hepatitis B, i.e. remains HBsAg-positive for more than 6 months (5,9,10). This chronic hepatitis B is usually of a very mild nature, with normal serum amino-transferases (9,10).

One child lost HBsAg in year 2. In our study the annual clearance rate of HBsAg was 3% (95% CI 0.4-22%); in the Taiwanese study describing the follow-up of 420 hepatitis B-infected children, the corresponding percentage was 0.6% (5). Two children became HBeAg-negative within 2 and 3 years respectively. In our study the annual clearance rate of HBeAg was 7% (95% CI 3-19%); in the Taiwanese study the corresponding percentage was less than 2% (6).

The HBsAg-positive children in the Taiwanese study were not vaccinated against hepatitis B, whereas the children in our study were. Data about the effect of hepatitis B vaccination on the course of hepatitis B after failed vaccination, have so far not been available. Examination of a greater number of HBsAg-positive children after failed vaccination would give us more insight into this.

What are possible causes for the failure of neonatal hepatitis B vaccination? To answer this question we determined HBeAg and quantitative HBV-DNA of the mothers, and the HLA type of hepatitis B-infected children. 118 of the 705 (17%) of the HBsAg-positive mothers were HBeAg-positive as well; maternal HBeAg

was positive of all 8 children infected with hepatitis B during the first year of their lives. Maternal HBV-DNA of the infected children (median 314, range 152-597 pg/ml) was significantly higher than in a control group of HBeAg-positive mothers of non-infected children (median 5, range 0-618 pg/ml) (11). We concluded that failure of immunisation was related to perinatal high maternal viral load (HBeAg, High HBV-DNA) and not to a genetically determined inability of the children to produce anti-HBs (11).

The results from our study might serve as a basis for a policy regarding the follow-up of young children with chronic hepatitis B. Recently some articles have been published about the administration of alpha-interferon to children with chronic hepatitis B (12,13). A Spanish study describes a virological (loss of HBV-DNA and HBeAg) and a biochemical (normalisation of ALAT) remission in over 40% of 24 children between ages 1.5 and 5, with chronic active hepatitis B, at 9 months after completion of a 6-months interferon therapy, against 17% remission in a control group were no alpha-interferon was administered (12). A Chinese study included asymptomatic children with normal serum transaminases; in this study no difference was found after 15 months in clearance percentage HBeAg of a control group (8%) and a group treated with interferon for 3 months (8%) (13). Therapy was tolerated well by children from both studies, side effects were mild and consisted of influenza symptoms, which disappeared after 2 weeks. Possible explanations for the different remission percentages can be found in the duration of the interferon therapy (Spanish study: 6 months, Chinese study: 3 months) and in the different selection of patients (Spanish study: children with chronic active hepatitis B (HBeAg-, HBV-DNA-positive) and raised serum amino-transferases, Chinese study: children with asymptomatic chronic hepatitis B with normal amino-transferases. For adults with chronic hepatitis B the possibility of spontaneous HBeAg sero-conversion is closely linked to the serum amino-transferases, with the possibility of HBeAg sero-conversion twice as high with interferon therapy (14,15). As far as we know there is at present no standard therapy for chronic hepatitis B (HBeAg-positive) children.

Given the possible serious complications of chronic hepatitis B (cirrhosis, hepato-cellular carcinoma) and given the availability of interferon, treatment of children with chronic hepatitis B and active viral replication (HBeAg-, HBV-DNA-positive) must be considered. However, the clinical picture is usually so mild as to warrant the moderately effective interferon therapy only in the most obvious cases. We think it advisable to perform, in addition to anamnesis, physical and virological examinations, annual hepatic tests, and in the case of abnormalities a periodic ultra-sound scan of the liver. Only for patients having abnormal amino-transferases lasting longer than a year, is there an increased risk of cirrhosis and is therapy medically indicated. In some cases the infectiousness of the patient may cause

great problems and in those cases therapy may be socially indicated. In order for a possible treatment to be started in the case of active viral replication and abnormal hepatic tests, check-ups should initially be carried out by a paediatrician.

Children with chronic hepatitis B without active viral replication (HBeAg- and HBV-DNA-negative) and with normal serum amino-transferases (ASAT, ALAT) do not qualify for anti-viral therapy and may be checked annualy by the family doctor to preclude reactivation.

Acknowledgement

We thank H. Ribbers, H. van Dijk, T.M.S. van de Laar en M. Bakker for their assistance in administration and Y.A.M. Weber for performing the laboratory tests.

This study was supported by a grant from the Praeventiefonds, the Netherlands (no. 28-760-2).

References

1. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; 2: 1099-102.
2. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'doye R. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986: 2: 1143-5.
3. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987; 257: 2612-6.
4. Hsu HY, Chang MH, Chen DS, Lee CY, Sung JL. Baseline seroepidemiology of hepatitis B virus infection in children in Tapei, 1984: a study just before mass hepatitis B vaccination program in Taiwan. J Med Virol 1986; 18: 301-7.
5. Hsu HY, Chang MH, Lee CY, Chen JS, Hsu HC, Chen DS. Spontaneous loss of HBsAg in children with chronic hepatitis B virus infection. Hepatology 1992; 15: 382-6.
6. Chang MH, Sung JL, Lee CY, Chen CJ, Chen JS, Hsu HY et al. Factors affecting clearance of hepatitis B e antigen in hepatitis B surface antigen carrier children. J Pediatr 1989; 115: 385-90.
7. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989; 83: 1041-7.
8. Kalbfleisch JD, Prentice RL, editors. The statistical analysis of failure time data. New York: John Wiley and sons, 1980.
9. Bortolotti F, Cadrobbi P, Crivellaro C, Guido M, Rugge M, Noventa F et al. Long-term outcome of chronic type B hepatitis in patients who acquire hepatitis B virus infection in childhood. Gastroenterology 1990; 99: 805-10.
10. Lok ASF, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive chinese children. Hepatology 1988; 8: 1130-3.
11. Canho del R, Grosheide PM, Schalm SW, Vries de RRP, Heijtink RA. Failure of neonatal hepatitis B vaccination: the role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates. J Hepatol: in press.
12. Ruiz-Moreno M, Rua MJ, Molina J, Moraleda G, Moreno A, Garcia-Aguado J et al. Prospective, randomized controlled trial of interferon- in children with chronic hepatitis B. Hepatology 1991; 13: 1035-9.
13. Lai CL, Lok ASF, Lin HJ, Wu PC, Yeoh EK, Yeung CY. Placebo-controlled trial of recombinant -interferon in chinese HBsAg carrier children. Lancet 1987; 877-80.
14. Janssen HLA, Berk L, De Man RA, Heijtink RA, Schalm SW. Antivirale behandeling met -interferon bij 100 patiënten met chronische hepatitis B. Ned Tijdschr Geneeskd 1992; 136: 835-9.
15. Krogsgaard K, Bindslev N, Christensen E, Craxi A, Schlichting P, Ring-Larsen H et al. The treatment effect of alfa interferon in chronic hepatitis B is independent of pretreatment variables: results of a meta-analysis of individual patient data. Submitted for publication.

Discussion:

Ten year hepatitis B vaccination for prevention of neonatal hepatitis B: what has been achieved, what needs to be done?

Discussion

Introduction

After 10 year of neonatal hepatitis B vaccination, it has become clear that hepatitis B vaccine is safe and highly immunogenic; it also is very effective in preventing perinatally transmitted hepatitis B. New insights in the risk of maternal infant transmission appear important for the evaluation of vaccination programs and open the way for improving the efficacy of neonatal hepatitis B vaccination, especially in countries which rely on screening pregnant women for HBV and provide passive-active vaccination for infants at risk. In addition, universal neonatal hepatitis B vaccination has been advised by the World Health Organization for all countries by 1997 (1). Many countries have already integrated or are moving towards integrating hepatitis B vaccination into their standard immunization program for neonates (EPI: Expanded Programme on Immunization). Results of large immunization trials with long-term follow-up may help countries in planning (mass) hepatitis B immunization programs.

Safety

No clinically important side effects have occurred with any of the licensed vaccines (plasma- and recombinant- vaccine). Incidental cases of central-nervous-system demyelination (2), uveitis (3), erythema nodosum (4) and systemic reactions (5) after hepatitis B vaccination have been reported, however a causal link between vaccination and these adverse effects has not been proved and has not been confirmed by others. With the introduction of recombinant-vaccine, the fears that other viruses (like HIV) might survive the manufacturing inactivation procedures, necessary for preparing plasma-vaccine, have wained. In our study, of 705 closely monitored infants, mild transient local symptoms were reported in 2.5% of infants vaccinated with 20 μg Engerix-B and in 15% of infants vaccinated with 5 and 10 μg HB-Vax-DNA hepatitis B vaccine (6,7), in agreement with earlier results published (8,9).

Immunogenicity

Neonates generally respond well to both plasma- and recombinant vaccine. An anti-HBs level of more than 10 IU/L is considered to provide protection against clinical important forms of hepatitis B (10); anti-HBs response of more than 100 IU/L implies long-lasting immunity (11). However the duration of immunity and the need for booster vaccinations have not yet been clearly established. 1-2% of

properly vaccinated infants of HBsAg-positive carrier mothers are not able to develop adequate amounts of anti-HBs for long-lasting immunity against hepatitis B. Both immunization and host factors affect the immune response to the hepatitis B vaccine.

Immunization factors includes e.g. type, dose and schedule of vaccine. Enhancement of immunogenicity can be obtained by higher doses of vaccine (12,13), starting with vaccination if the infant is at an older age (14) and by using plasma- instead of recombinant- vaccine (15). In our study, anti-HBs levels measured after 12 months of follow-up were approximatively two times higher in infants receiving 3 doses of 10 μg plasma-vaccine than in infants receiving only half of this dosage (16). This difference in anti-HBs response was no longer significant after 36 months of follow-up. In our study with recombinant-vaccine, there was no significant difference in immunogenicity between 10 and 5 μg vaccine after 12 months of follow up (7). We confirmed the finding that plasma-vaccine yields higher anti-HBs levels than recombinant-vaccine (16). Anti-HBs levels after immunization of infants at 3 months (concomitant with DTPP vaccination) were significantly higher than in infants who were vaccinated at birth (17,6), probably related to a more mature immune system of infants at 3 months of age. The implications of this finding are not clear, it may become of importance if follow up results beyond 5-10 years become available. The observation that efficacy and immunogenicity are excellent when vaccination is started several months after birth, allows incorporation of hepatitis B vaccine in the routine infant immunization programme. Incorporation of hepatitis B vaccine into the existing Expanded Programme on Immunization (EPI) could reduce the number of doctor visits and could enhance compliance to both programmes. If protection against hepatitis B infection depends on persistence of antibody in a major way, then a strong argument could be made for adoption of schedules that maximize anti-HBs levels. Otherwise, adequate priming of the immune system, reflected by initial anti-HBs level of more than 100 IU/L after completion of vaccination is the goal of prevention (18). For the time being, until more long-term follow up studies are present, it seems advisable to achieve an initial anti-HBs response of more than 100 IU/L after vaccination for the prevention of late chronic hepatitis B.

Infant immune responsiveness may depend on genetic causes (19). In our study, with only a small number of individuals, we found that 50% of (noninfected) low responders (anti-HBs less than 10 IU/L) were positive for HLA-DR3. None of the infants that became HBsAg carriers was HLA-DR3 positive, suggesting no genetic cause for the failure of hepatitis B immunization (20,21). Impaired immune responses to hepatitis B vaccine have been described in children undergoing hemodialysis (22), in children with malignancies (23) and in HIV

infected infants (24). In our study group, all (noninfected) low responders were in excellent health and diseases, that lower the activity of the immune system, were not observed. These low responders were not absolute low responders, since all of them developed protective anti-HBs levels after hepatitis B revaccination in their second year of life (25). We found no impaired immune response to hepatitis B vaccination in preterm infants in comparison with full term infants (26), in contrast with others (27).

Efficacy

The main goal of hepatitis B immunization is the prevention of the HBsAg carrier state and the sequalae, cirrhosis and hepatocellular carcinoma. Protective efficacy of more than 90% of passive-active immunization with plasma- and recombinant- hepatitis B vaccine has been described in programs for neonates (28-31,14). In our study, the Protective Efficacy Rate (PER) at 12 month of age for infants of HBeAg positive carrier mothers was 92%, for infants of HBeAg negative mothers the PER was 100%. No effect on PER was found for timing of active immunization, number of doses of HBIg and the type of vaccine (16), confirming previous observations (28,31). We found no need for a second dose of HBIg in combination with late-active immunization, but this finding has not been investigated by others. However, we found a major clinically relevant factor that influenced the PER. PER in the groups of infants with maternal HBV-DNA levels less than 150 pg/ml was 100% and significantly higher than 68% in the group with maternal HBV-DNA level of more than 150 pg/ml.

To verify the finding that the protective efficacy rate at 12 months of age is markedly influenced by maternal HBV-DNA levels, we reanalyzed the protective efficacy rate at 12 months of age according to quantified maternal HBV-DNA levels, in the Hong Kong neonatal hepatitis B vaccination programme (30,32,33) (table 1). In infants with maternal HBV-DNA levels of less than 5 pg/ml, no persistent HBsAg positivity at 12 months was detected, in agreement with our study. It was also confirmed that infants with maternal HBV-DNA levels of more than 150 pg/ml were at high risk for hepatitis B (25-50% of infants became persistent HBsAg positive) in spite of immunization. In infants with maternal HBV-DNA between 5-150 pg/ml, 15-28% of infants became HBsAg positive in the Hong Kong study. In the Dutch study no persistent HBsAg positivity was found. These results strongly support the concept that the level of maternal HBV-DNA is a major factor influencing the outcome of hepatitis B immunization. The absence of HBsAg carriers in the group with moderately high maternal HBV-DNA in our study, in comparison to 15-28% carrier rate in the Hong Kong study needs additional discussion.

Table 1. Failure of perinatal hepatitis B immunization in infants of HBeAg-carrier mothers, according to maternal HBV-DNA levels, in Hong-Kong and the Netherlands.

HBV-DNA (pg/ml)		Number of persistently HBsAg positive infants at 12 month (%)				
		Placebo	vaccine only*	HBIg/ vaccine*	HBIg/ vaccine**	mult.HBIg/ vaccine***
		group IV	group III	group II	group 0	group I
		Hong Kong	Hong Kong	Hong Kong	The Neth.	Hong Kong
n=78	< 5	0/9 (0%)	0/16 (0%)	0/13 (0%)	0/24 (0%)	0/16 (0%)
n=181	5-150	25/31 (81%)	11/40 (28%)	7/47 (15%)	0/24 (0%)	1/39 (3%)
n=48	≥ 150	5/7 (71%)	4/8 (50%)	1/4 (25%)	7/24 (29%)	0/5 (0%)
total		30/47 (64%)	15/64 (23%)†	8/64 (13%)	7/72 (10%)[a]	1/60 (2%)††

* HBIg single dose of 100 IU at birth (CLB, The Netherlands), 4 doses of 3 μg plasma-derived hepatitis B vaccine (CLB)
** HBIg 1 or 2 doses at birth (and 3 month) of 200-300 IU (CLB), 4 doses of 10 μg plasma-derived hepatitis B vaccine (MSD) or 20 μg recombinant-DNA hepatitis B vaccine (SKB)
*** HBIg 7 monthly doses from birth onwards of 100 IU (CLB), 4 doses of 3 μg plasma-derived hepatitis B vaccine (CLB)
† 1 infant became persistently HBsAg+ after 12 months of age
†† 4 infants became persistently HBsAg+ after 12 months of age
[a] maternal HBV-DNA in 1 persistent HBsAg+ infant not available

We left group I out of consideration, because 4 of 5 infected infants became persistent HBsAg positive after 12 months of age, possible due to horizontal hepatitis B infection.

Since the rate of intrauterine infection is estimated to be only 1-2% (30), the difference in HBV carrier rate is therefore likely due to differences in intervention (HBIg and vaccine) or to differences in maternal fetal transfusion during labour. Infants became persistent HBsAg positive after month 12 in the Hong Kong study, but not in our study. The dose of HBIg and vaccine might therefore be important for the prevention of late hepatitis B infection, in infants at risk for hepatitis B. Lee et al. found that the number of infants with vertically transmitted HBV could significantly reduced by performing a Caesarean section, to reduce the amount of maternal fetal transfusion during labour (34).

Recently, hepatitis B "escape mutants", lacking the "a" epitope on the viral envelope were found in vaccinated infants (35). The facts that we did not see a coexistence of HBsAg and anti-HBs positivity in 8 of 9 HBsAg positive infants and full neutralization of HBsAg by HBIg was obtained in all HBsAg positive children, make the presence of surface antigen variants in our study population highly unlikely.

Recommendations for hepatitis B immunization protocols

EPI

Hepatitis B vaccine can and should be incorporated into existing vaccination programs for neonates (EPI: Expanded Programme on Immunization). A multivalent vaccine should be developed as rapidly as possible in order to reduce the number of injections and the number of doctor visits.

Screening

For countries with a hepatitis B screening program for pregnant women, in particular for countries which can afford high level individual care, assessment of the quantitative HBV-DNA level should be performed in all HBsAg-positive women that are also positive for HBeAg. Development of a cheap HBV-DNA quantitation assay is highly desirable.

HBIg/vaccine/additional measures

For countries with hepatitis B screening program for pregnant women, one dose of HBIg at birth in combination with delayed-active immunization, incorporated in EPI, appears the strategy of choice in view of efficacy, compliance and costs. In countries with high endemicity of hepatitis B, which can not afford screening, global vaccination without HBIg at birth might be the policy. During the last few years, the price of vaccines in developing countries has fallen to the point where widespread use of hepatitis B vaccine in EPI is feasible. There is need for confirmation of findings in large scale programmes.

It is advised to offer at least 300 IU of HBIg and full dose of vaccine to neonates of HBsAg carrier mothers with maternal HBV-DNA level between 5 and 150 pg/ml.

Additional measures are required for infants at highly risk with maternal HBV-DNA level of more than 150 pg/ml; to enhance the capture of HBV during the first days of life, the use of a higher dose of HBIg at birth should be studied. To reduce maternal fetal transfusion during labour, the role of Caesarean section should be reevaluated in a schedule using current doses of HBIg and vaccine.

Efficacy of HBIg dosage and vaccine schedules should be assessed in neonates with defined maternal HBV-DNA levels. Vaccine types and schedules should be used, providing adequate immune response beyond the first year. Such vaccine programmes are in general defined by initial anti-HBs response of more than 100 IU/L in more than 95% of vaccinees.

Preterm infants

Infants with a gestational age of less than 37 weeks should be vaccinated, at similar age as term infants, without correction for their shortened gestational age.

Infants with low/no response to hepatitis B vaccine

Infants with an inadequate anti-HBs response after primary vaccination and who remained HBsAg negative, should receive additional hepatitis B vaccinations in their second year of life.

References

1. World Health Organization, Expanded Programme on Immunization, Global Advisory Group. Weekley Epidemiolocal Record 1992; 3: 11-16.
2. Herroelen L, De Keyser J, Ebinger G. Central-nervous-system demyelination after immunisation with recombinant hepatitis B vaccine. Lancet 1991; ii: 1174-5.
3. Fried M, Conen D, Conzelmann M, Steineman E. Uveitis after hepatitis B vaccination. Lancet 1987; ii: 631-2.
4. Di Giusto CA, Bernhard JD. Erythema nodosum provoked by hepatitis B vaccine. Lancet 1986; ii: 1042.
5. Snider GB, Gogate SA. A possible systemic reaction to hepatitis B vaccine. JAMA 1985; 253: 1260-61.
6. Canho del R, Grosheide PM, Voogd M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of a full dose (20 μg) of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes. J Med Virol 1993; 41: 30-4.
7. Canho del R, Grosheide PM, Voogd M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of two different dosages (10 μg and 5 μg) of recombinant DNA hepatitis B vaccine in healthy neonates. Submitted for publication.
8. Poovorawan Y, Sanpavat S, Pongpunlert W, Chumdermpadetsuk S, Chitinand S, Sakulramrung R et al. Immunogenicity and protective efficacy of a yeast-derived hepatitis B vaccine in neonates. In: Coursaget P, Tong MJ (eds): `Progress in Hepatitis B Immunization'. London, Paris: John Libbey Eurotext 1990, pp 371-7.
9. Dandolos E, Roumeliotou-Karayannis A, Richardson SC, Papaevangelou G. Safety and immunogenicity of a recombinant hepatitis B vaccine. J Med Virol 1985; 17: 57-62.
10. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. Hepatology 1981; 5: 377-85.
11. Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986; 315: 209-14.
12. Goh KT, Tan KL, Kong KH, Oon CJ, Chan SH. Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: a four-year follow up study. Bulletin of the World Health Organization 1992; 70 (2): 233-9.
13. Milne A, Moyes CD, Allwood GK, Pearce NE, Krugman S. Antibody responses to recombinant, yeast-derived hepatitis B vaccine in teenage New Zealand children. NZ Med J 1988; 101: 67-9.
14. Stevens CE, Toy PT, Taylor PE, Lee Th, Yip HY. Prospects for control of hepatitis B virus infection: Implications of childhood vaccination and long-term protection. Pediatr 1992; 90: 170-3.
15. Jilg W, Lorbeer B, Schmidt M, Wilske B, Zoulek G, Deinhardt F. Clinical evaluations of a recombinant hepatitis B vaccine. Lancet 1984; ii: 1174-5.
16. Canho del R, Grosheide PM, Heijtink RA, Hop WCJ, Mazel JA, Gerards LJ, Gast de GC, Schalm SW, Dutch study group Prevention Neonatal Hepatitis B. Ten-year neonatal hepatitis B vaccination program, The Netherlands, 1982-1992: Protective efficacy according to maternal serum levels of HBV-DNA and long-term immunogenicity. Submitted for publication.
17. Grosheide PM, Canho del R, Heijtink RA, Nuijten ASM, Zwijnenberg J, Bänffer JRJ et al. Passive-active immunization of infants of hepatitis B e-antigen positive mothers: comparison of the efficacy of early and delayed active immunization. Am J Ped Inf Dis: in press.
18. Jilg W, Schmidt M, Deinhardt F. Decline of anti-HBs after hepatitis B vaccination and timing of revaccination. Lancet 1990; i: 173-4.
19. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321: 708-12.
20. Canho del R, Vries de RRP, Schalm SW. Human Leucocyte Antigens (HLA) in neonates with an inadequate response to hepatitis B vaccination. Vaccine 1993; 11: 983.
21. Canho del R, Grosheide PM, Schalm SW, Vries de RRP, Heijtink RA. Failure of neonatal

hepatitis B vaccination: the role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates. J Hepatol 1994: in press.
22. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing hemodialysis. Acta Paediatr Scand 1985; 74: 213-8.
23. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H et al. Hepatitis B vaccination and immune response in children with malignant diseases. Eur J Pediatr 1985; 144: 160-3.
24. Zuin G, Principi N, Tornaghi R, Paccagnini S, Re M, Massironi E et al. Impaired response to hepatitis B vaccine in HIV infected children. Vaccine 1992; 10: 857-60.
25. Canho del R, Schalm SW, Heijtink RA. Hepatitis B revaccination of neonates with inadequate response after primovaccination. Vaccine 1992: 10: 69.
26. Canho del R, Grosheide PM, Gerards LJ, Heijtink RA, Schalm SW. Hepatitis B vaccination and preterm infants. Ped Inf Dis J 1993; 12: 407: 8.
27. Lau YL, Tam AYC, Ng KW, Tsoi NS, Lam B, Lam P et al. Response of preterm infants to hepatitis B vaccine. J Pediatr 1992; 121: 962-5.
28. Beasley RP, Hwang LY, Lee GC, Lan C, Roan C, Huang F et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-102.
29. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'Doye R et al. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986; ii: 1143-5.
30. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY et al. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984; i: 921-6.
31. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV et al. Yeast-recombinant hepatitis B vaccine: Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B transmission. JAMA 1987; 257: 2612-16.
32. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP et al. Prevention of maternal-infant hepatitis B virus transmission by immunization: The role of serum hepatitis B virus DNA. Hepatol 1986; 6: 369-73.
33. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW . Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989; i: 406-10.
34. Lee SD, Lo KJ, Tsai YT, Wu JC, Wu TC, Yang ZL et al. Role of Caeasarean section in prevention of mother-infant transmission of hepatitis B virus. Lancet 1988; ii: 833-4.
35. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E et al. Vaccine-induced escape mutant of hepatitis B virus. Lancet 1990; 336: 325-29.

Chapter 12

Summary
Samenvatting
Dankwoord
Curriculum vitae

Summary

Chapter 2

Chapter 2 describes the protective efficacy of passive-active immunization in 80 high-risk infants after a follow-up of 5 years. Infants received either early active immunization starting at birth in combination with HBIg at birth (group A) or late active immunization starting at 3 months of age in combination with HBIg at 0 and 3 months of age (group B). During the study period 3 of 35 infants in group A and 3 of 37 infants in group B became HBsAg carriers, corresponding to an incidence of infection of 9% and 8%, respectively. Subclinical infections were diagnosed in 4 infants in each group. Late active-immunization starting at 3 months of age appears to provide similar protective efficacy as active immunization starting at birth when combined with hepatitis B immune globulin at 0 and 3 months of age. Late active hepatitis B immunization starting at 3 months of age could be given at the same time as DTPP immunization. Incorporation of hepatitis B immunization in the existing DTPP program may increase compliance and decrease costs.

Chapter 3

Chapter 3 describes the immunogenicity of a full dose of recombinant DNA yeast-derived hepatitis B vaccine (Engerix-B) in healthy neonates of HBsAg negative mothers. Three candidate vaccinations schemes were compared. Neonates received 4 doses of hepatitis B vaccine, starting either at month 3 or at birth. Another group of neonates received hepatitis B vaccine according to a three-dose scheme starting at birth. The vaccine was highly immunogenic irrespective of vaccination scheme; all infants developed anti-HBs levels of more than 10 IU/L, 97% more than 100 IU/L. The immunogenicity of hepatitis B vaccine administered in the four-dose scheme starting at birth was significantly higher than in the three-dose scheme starting at birth. Hepatitis B vaccination according to the four-dose scheme started at month 3 produced significantly higher antibody levels in comparison to the four-dose scheme started directly after birth.

Chapter 4

Chapter 4 describes the immunogenicity of one half and a full dosage of recombinant DNA yeast-derived hepatitis B vaccine (HB-Vax-DNA) in healthy neonates of HBsAg negative mothers. Two candidate dosages of the vaccine were

compared. Neonates received four doses of one half (5 μg) or a full (10 μg) dosage of vaccine, concomitant with DTP-Polio immunization. The vaccine was highly immunogenic not related to dosages of vaccine; all infants developed anti-HBs levels of more than 10 IU/L and 99% more than 100 IU/L.

Chapter 5

Chapter 5 describes the efficacy and long-term immunogenicity of the ten-year neonatal hepatitis B vaccination program 1982-1992. 705 infants born to HBsAg positive mothers entered the Dutch neonatal hepatitis B vaccination and received passive-active hepatitis B immunization, according to 6 schedules, varying in time of onset vaccination, dose of HBIg and type and dose of vaccine. 118 (17%) of the mothers were also HBeAg positive. During follow up, 9 infants became HBsAg carriers; all born to HBeAg positive mothers within the first year and another child, born to HBeAg negative mother at the age of 5 years. Protective Efficacy Rate (PER) of passive-active hepatitis B immunization at 12 months follow up in the total group was 92% with no significant difference in groups starting at birth or at 3 months; groups receiving one or two doses of HBIg; groups receiving plasma- derived or recombinant- vaccine. PER at month 12 in the group with maternal HBV-DNA levels < 150 pg/ml was 100% and significantly higher than 68% for the group with HBV-DNA levels ≥ 150 pg/ml. After 5 years of follow up, the group with active immunization starting at 3 months had significantly less infants with anti-HBs levels less than 10 IU/L (2%) than the corresponding group starting at birth (15%). The GMT anti-HBs (anti-HBs ≥ 10 IU/L) was significantly higher in the group, starting at 3 months of age with plasma vaccine than in the corresponding group starting at birth and also higher than in the corresponding group, using recombinant vaccine. This program showed that hepatitis B vaccine is highly effective and immunogenic in the prevention of neonatal hepatitis B. Evaluation of vaccination programs according maternal HBV-DNA levels is important for improving results of intervention for those infants at highest risk of vertically transmitted hepatitis B.

Chapter 6

Chapter 6 The immune response after passive-active hepatitis B immunization of 44 preterm neonates (gestational age of less than 37 weeks) was compared with the immune response of 829 full term infants. No significant differences between preterm infants and full term infants in the percentages with anti-HBs titers more than 10 IU/L and more than 100 IU/L were found. The percentages of children with anti-HBs titers more than 10 IU/L and more than 100 IU/L who

started vaccination at birth were lower than the corresponding percentages of infants who started at month 3, but they were similar for preterm and full term infants. There was no significant difference in immune response after completion of the primary vaccinations between preterm infants weighing less and those weighing more than 2000 grams. The anti-HBs titers at month 3 of 2 preterm infants receiving exchange transfusions, were significantly lower than for infants without exhange transfusions. Replacement of plasma shortly after passive immunization could theoretically lead to inadequate protection against hepatitis B infection. Administration of additional doses of HBIg, after exhange transfusion, would probably prevent the risk of hepatitis B infection. Although there was only one child with a gestational age of less than 28 weeks in our study, we support the recommendation of the American Academy of Pediatrics to administer hepatitis B vaccine as well as diphtheria, tetanus and pertussis vaccine to preterm infants at the appropriate chronological age.

Chapter 7

Chapter 7 describes the results of additional hepatitis B vaccination of infants, who had a non-detectable or a weak response after primovaccination (anti-HBs $\leq$ 10 IU/L) and no signs of hepatitis B infection. Nine infants received 3-4 additional doses of plasma-derived (10 μg) or recombinant-DNA (20 μg) vaccine in their second year of life. After revaccination all infants showed an anti-HBs response above 10 IU/L; 7 children developed more than 50 IU/L and 4 of them more than 100 IU/L. Since many of these children remain at risk for hepatitis B infection due to family contacts, it seems important to determine anti-HBs levels after vaccination and to offer additional doses of hepatitis B vaccine to HBsAg negative children with an anti-HBs level less than 10 IU/L.

Chapter 8

Chapter 8 showed the HLA phenotypes of the 8 noninfected low responders (HBsAg negative, anti-HBs less than 10 IU/L) and the 8 infected nonresponders (HBsAg positive, anti-HBs negative) to hepatitis B vaccine. HLA-DR3 was present in 4 of the 8 noninfected low responders and in none of the 8 infected nonresponders. Two noninfected low responders were probably homozygous for HLA-DR3. This study suggested that the HLA-DR3 haplotype plays a role in the low responsiveness to hepatitis B vaccination in noninfected neonates but not in HBsAg positive neonates, in which vaccination failed.

Chapter 9

Chapter 9 describes the results of studies to hepatitis B immunization failure. In order to determine whether failure of the hepatitis B vaccination was due to perinatal maternal high-level viraemia or genetically determined infant non-responsiveness to the vaccine, we measured HBeAg and HBV-DNA levels in maternal serum and determined the HLA type of the 8 infected nonresponders (group 1). Controls included 14 infants with a normal anti-HBs response 1 year after vaccination, noninfected responders (group 2), and the 8 noninfected low responders (group 3). All infants in group 1 and 2 were born of HBeAg carrier mothers, those in group 3 of HBeAg-negative mothers. HBV-DNA levels in maternal serum in group 1 were significantly higher than in group 2 and 3. HLA B8 and DR3 were not found in group 1 (chapter 8). It is believed that intra-uterine HBV infection occurs in 1-2% of infants of HBeAg positive HBV carrier mothers. For a similar incidence in our study, intra-uterine infections can be expected in 2 infants of 118 HBeAg carrier mothers. The remaining causes of failure of hepatitis B vaccination appears to be related not to genetic nonresponsiveness of infants but rather to perinatal maternal high-level viraemia. HBV-DNA assay of HBeAg-positive mothers may identify those infants in need of additional action to lower the risk of vertically transmitted HBV infection.

Chapter 10

Chapter 10 describes virological, clinical and biochemical characteristics of HBV in infants, who became hepatitis B infected, in spite of passive-active hepatitis B immunization. Nine of 705 vaccinated infants became positive for HBsAg. Median follow up was 5 years (range 3-8 years). Eight of the 9 infants were also positive for HBeAg. At the end of the follow up, 1 child lost HBsAg and 2 children lost HBeAg. Only 1 child experienced a symptomatic hepatitis B infection with raised transaminase levels. The other 8 infants with chronic hepatitis B were not symptomatic and were in excellent health. Transaminase levels were ever normal in 7 infants. The conclusion was that most of the infants, who became HBsAg positive, in spite of passive-active hepatitis B immunization, developed a chronic hepatitis B infection, without clinical and biochemical dysfunctions. On the basis of these findings and recent literature, concerning possible therapy, guidelines were given for the follow up of children with chronic hepatitis B. Antiviral therapy should be considered for chronically HBV infected children with active viral replication (HBeAg and HBV-DNA positive) and raised transaminase levels for 1 year.

Samenvatting

Hoofdstuk 2

Hoofdstuk 2 beschrijft de effectiviteit van passieve-actieve immunisatie van 80 pasgeborenen met een groot risico op hepatitis B na een observatie-periode van 5 jaar. Na randomisatie, ontvingen kinderen vroege actieve immunisatie, de eerste dosis vaccin in combinatie met HBIg, direct na de geboorte (groep A) of late actieve immunisatie, de eerste dosis vaccin op 3 maanden in combinatie met HBIg direct na de geboorte en op de leeftijd van 3 maanden (groep B). Drie van 35 kinderen in groep A en 3 van 37 kinderen in groep B werden HBsAg positief. Subklinische infecties werden in 4 kinderen van elke groep gediagnostiseerd. De effectiviteit van late actieve immunisatie, gecombineerd met HBIg op maand 0 en 3, was gelijk aan de effectiviteit van vroege actieve immunisatie. Late actieve hepatitis B immunisatie, waarbij de eerste dosis op de leeftijd van 3 maanden wordt gegeven, kan tegelijkertijd met de DKTP vaccinatie plaatsvinden. Incorporatie van hepatitis B vaccin in het bestaande DKTP programma kan de kosten verminderen en de bereidheid tot deelname aan het programma vergroten.

Hoofdstuk 3

Hoofdstuk 3 beschrijft de immunogeniciteit van een volwassen dosis recombinant DNA hepatitis B vaccin (Engerix-B) in gezonde pasgeborenen van HBsAg negatieve moeders. Drie vaccinatie schema's werden vergeleken. Bij de eerste groep kinderen werd de vaccinatie direct na de geboorte gestart en 3 maal herhaald. Bij een tweede groep kinderen werd eveneens direct na de geboorte gestart en de vaccinatie slechts 2 maal herhaald. Bij een derde groep kinderen werd op de leeftijd van 3 maanden de eerste vaccinatie gegeven en werd deze 3 maal herhaald. De immunogeniciteit van het vaccin was groot, onafhankelijk van het gebruikte schema; alle kinderen produceerden een anti-HBs titer van meer dan 10 IE/L, 97% meer dan 100 IE/L. De immunogeniciteit van hepatitis B vaccin in het schema met 4 doses vanaf de geboorte was significant groter dan van het schema met 3 doses vanaf de geboorte. Hepatitis B vaccinatie volgens het 4 doses schema, waarbij de eerste dosis op de leeftijd van 3 maanden wordt gegeven, gaf hogere anti-HBs titers dan het 4 doses schema waarbij de eerste dosis direct na de geboorte werd gegeven.

Hoofdstuk 4

Hoofdstuk 4 beschrijft de immunogeniciteit van een halve en een hele dosis recombinant DNA hepatitis B vaccin (HB-Vax-DNA) bij gezonde kinderen van HBsAg negatieve moeders. Twee mogelijke schema's werden vergeleken. Kinderen ontvingen 4 maal een halve (5 μg) of een hele (10 μg) dosis vaccin, tegelijkertijd met de DKTP vaccinatie. De immunogeniciteit van het vaccin was groot en niet gerelateerd aan de dosis; alle kinderen ontwikkelden anti-HBs titers van meer dan 10 IE/L en 99% meer dan 100 IE/L.

Hoofdstuk 5

Hoofdstuk 5 beschrijft de effectiviteit en lange-termijn immunogeniciteit van het 10-jaar neonatale hepatitis B vaccinatie programa 1982-1992. 705 kinderen van HBsAg positieve moeders namen deel aan het programma en ontvingen passieve-actieve hepatitis B immunisatie, variërend in tijdstip van starten vaccinatie, dosis HBIg en type en dosis vaccin. 118 (17%) van de moeders waren ook HBeAg positief. Gedurende de observatie-periode werden 9 kinderen HBsAg positief; 8 kinderen, allemaal van HBeAg positieve moeders, in het eerste levensjaar en 1 kind van een HBeAg negatieve moeder, op de leeftijd van 5 jaar. De effectiviteit van passieve-actieve hepatitis B immunisatie na een observatie-duur van 12 maanden bedroeg 92% in de totale groep; geen significante verschillen werden gevonden in de groepen waarbij de eerste dosis werd gegeven direct na de geboorte of op de leeftijd van 3 maanden; in de groepen die 1 of 2 doses HBIg ontvingen of in de groepen die plasma of recombinant vaccin ontvingen. De effectiviteit op maand 12 in de groep met maternale HBV-DNA < 150 pg/ml was 100% en significant groter dan de 68% in de groep met HBV-DNA ≥ 150 pg/ml. Na een observatie-periode van 5 jaar had de groep die de eerste dosis vaccin op de leeftijd van 3 maanden ontving significant minder kinderen met anti-HBs titers minder dan 10 IE/L (2%) dan de overeenkomstige groep die de eerste dosis vaccin direct na de geboorte ontving (15%). GMT anti-HBs (anti-HBs ≥ 10 IE/L) was siginificant hoger in de groep met de eerste dosis plasma vaccin op de leeftijd van 3 maanden dan in de overeenkomstige groep met de eerste dosis vaccin direct na de geboorte en ook hoger dan de overeenkomstige groep met recombinant vaccin. In dit programma was de effectiviteit van hepatitis B vaccin groot in de preventie van neonatale hepatitis B. Vaccinatie programma's evalueren aan de hand van maternale HBV-DNA lijkt zinvol voor het verbeteren van resultaten van interventie bij kinderen met het hoogste risico op verticaal verworven hepatitis B.

Hoofdstuk 6

Hoofdstuk 6 De immuunrespons na passieve-actieve hepatitis B immunisatie van 44 praematuren (zwangerschapsduur minder dan 37 weken) werd vergeleken met de immuunrespons van 829 à terme geboren kinderen. Er werden geen significante verschillen gevonden tussen praematuren en à terme geboren kinderen in de percentages kinderen met anti-HBs titers meer dan 10 IE/L en meer dan 100 IE/L. Er was geen significant verschil in immuunrespons tussen praematuren met een gewicht van minder dan 2000 gram of van meer dan 2000 gram. De anti-HBs titers op maand 3 van 2 praematuren die wisseltransfusies toegediend kregen, waren significant lager dan die van kinderen zonder wisseltransfusies. Het wegnemen van plasma kort na toediening van HBIg zou theoretisch kunnen leiden tot onvoldoende protectie tegen hepatitis B. Het toedienen van extra HBIg aan kinderen die wisseltransfusies krijgen zou het risico op hepatitis B kunnen verminderen. Alhoewel er slechts één kind een zwangerschapsduur van minder dan 28 weken had, ondersteunt deze studie vooralsnog het advies van de Gezondheidsraad om hepatitis B vaccinatie van praematuren te starten op de kalenderleeftijd, zonder te corrigeren voor de vroeggeboorte.

Hoofdstuk 7

Hoofdstuk 7 beschrijft de resultaten van additionele hepatitis B vaccinatie van kinderen, met een niet-detecteerbare of zwakke immuunrespons na de eerste vaccinaties (anti-HBs ≤ 10 IE/L) en die geen hepatitis B hadden. Negen kinderen ontvingen 3-4 additionele doses plasma (10 μg) of recombinant (20 μg) vaccin in hun tweede levensjaar. Alle kinderen hadden anti-HBs titers van meer dan 10 IE/L na revaccinatie, 7 kinderen meer dan 50 IE/L en 4 meer dan 100 IE/L. Omdat veel van deze kinderen een verhoogd risico op hepatitis B houden door familiecontacten, lijkt het belangrijk om de anti-HBs titers na vaccinatie te bepalen en om additionele doses hepatitis B vaccin aan te bieden aan HBsAg negatieve kinderen met een anti-HBs titer kleiner dan 10 IE/L.

Hoofdstuk 8

Hoofdstuk 8 laat de HLA phenotypes van de 8 niet-geïnfecteerde lage responders (HBsAg negatief, anti-HBs minder dan 10 IE/L) en van de 8 geïnfecteerde niet-responders (HBsAg positief, anti-HBs negatief) op vaccinatie zien. HLA-DR3 was in 4 van de 8 niet-geïnfecteerde lage responders en in géén van de 8 geïnfecteerde niet-responders aanwezig. Twee niet-geïnfecteerde lage responders waren waarschijnlijk homozygoot voor HLA-DR3. Deze studie suggereert dat het

HLA-DR3 haplotype een rol speelt in de lage immuunrespons op hepatitis B vaccinatie in niet-geïnfecteerde neonaten maar niet in HBsAg positieve kinderen, waarbij vaccinatie faalde.

Hoofdstuk 9

Hoofdstuk 9 beschrijft de resultaten van onderzoek naar oorzaken van falen hepatitis B vaccinatie. Om te bepalen of perinatale maternale hoge virale druk of genetisch bepaald onvermogen van het kind om op vaccinatie te reageren de oorzaak was van falen van hepatitis B vaccinatie, werden het HBeAg en HBV-DNA in maternale sera en het HLA-type van de 8 geïnfecteerde niet-responders bepaald (groep 1). Als controles fungeerden 14 kinderen met een normale anti-HBs respons 1 jaar na vaccinatie, niet-geïnfecteerde responders (groep 2) en de 8 niet-geïnfecteerde lage responders (groep 3). Alle moeders van de kinderen van groep 1 en 2 waren HBeAg positief, van de kinderen van groep 3, HBeAg negatief. HBV-DNA in maternaal serum van groep 1 was significant hoger dan van groep 2 en 3. HLA B8 en DR3 werden niet in groep 1 gevonden (hoofdstuk 8). Intra-uterine infecties zouden in 1-2% van de kinderen van HBeAg positieve moeders met chronische hepatitis B voorkomen. Bij een overeenkomstige incidentie in deze studie, werden ten hoogste 2 intra-uterine geïnfecteerde kinderen, van 118 HBeAg positieve moeders, verwacht. De overige oorzaken van falen van hepatitis B vaccinatie lijkt niet gerelateerd aan genetische bepaald onvermogen van het kind om op vaccinatie te reageren maar aan perinatale maternale hoge virale druk. Het bepalen van HBV-DNA bij HBeAg positieve moeders kan die kinderen identificeren met een hoog risico op verticaal verkregen hepatitis B.

Hoofdstuk 10

Hoofdstuk 10 beschrijft het virologische, klinische en biochemisch beloop van de hepatitis B infectie in kinderen, die ondanks passieve-actieve hepatitis B immunisatie toch HBsAg positief werden. Negen van de 705 gevaccineerde kinderen werden HBsAg positief. De mediane observatie-periode bedroeg 5 jaar (spreiding 3-8 jaar). Acht van de 9 kinderen waren tevens HBeAg positief. Aan het eind van de observatie-periode had 1 kind HBsAg en 2 HBeAg verloren. Slechts 1 kind maakte een symptomatische hepatitis B infectie met verhoogde serumtransaminasen door. De overige 8 kinderen met chronische hepatitis B waren zonder symptomen en verkeerden in goede algemene gezondheid. De serumtransaminasen van 7 kinderen waren immer normaal. De conclusie was dat het merendeel van de kinderen die HBsAg positief werden, ondanks passieve-actieve hepatitis B immunisatie, een chronische hepatitis B ontwikkelden, zonder klinische

en biochemische afwijkingen. Op grond van deze bevindingen en recente literatuur, betreffende mogelijke therapie, worden richtlijnen voor de begeleiding van kinderen met chronische hepatitis B gegeven. Antivirale therapie voor kinderen met chronische hepatitis B en actieve virale replicatie (HBeAg en HBV-DNA positief) met verhoogde serumaminotransferasen gedurende 1 jaar, moet overwogen worden.

Dankwoord

Veel mensen hebben bijgedragen aan dit onderzoek. Het is een eer voor mij hen op deze wijze te kunnen bedanken.

In het bijzonder mijn promotor Prof. Dr. Solko Schalm, die mij met enthousiasme de weg heeft leren verkennen in de wetenschappelijke wereld. Zijn kritische, inventieve en perfectionistische werkwijze maakt hem voor mij een echte leermeester.

Ruud Heijtink ben ik zeer erkentelijk voor zijn steun op virologisch terrein en het lezen van de vele versies manuscripten.

"Yvonne, Coby, Gerrie en Hans van het Lab" wil ik bedanken voor het nauwgezet uitvoeren van alle hepatitis B bepalingen voor dit onderzoek.

Zonder de "werkgroep Preventie Neonatale Hepatitis B"* was dit boekje er nooit gekomen. Veel dank ben ik verschuldigd aan talloze Rotterdamse, Utrechtse en Twentse medewerkers aan dit project.

Met veel respect en bewondering wil ik alle kinderen en hun ouders die aan dit onderzoek hebben deelgenomen bedanken.

Ruim 2 jaar werken in een kamer zonder ramen lukt alleen met de gezelligheid en steun van alle collega's en medewerksters op de afdeling. De rust op de Interne Geneeskunde II op woensdag-middag zal inmiddels zijn wedergekeerd zonder het baby- prik- spreekuur.

Jan Boot dank ik voor de computer-ondersteuning. Ondanks deze fluitende onmisbare hulp, loop ik nog steeds enkele versies van de diverse programma's achter en ben ik nog steeds bang voor de muis.

Ir. Wim C. Hop dank ik voor de talrijke adviezen op het gebied van de statistiek.

Heel blij ben ik dat Marja Bakker en mijn vader paranimfen willen zijn.
Tenslotte wil ik mijn familie, vrienden en nieuwe collega's in het JKZ bedanken voor de ondersteuning bij de totstandkoming van dit proefschrift en de relativering hiervan.

* Leden van de werkgroep Preventie Neonatale Hepatitis B:

S.W. Schalm, R. del Canho, P.M. Grosheide, afdeling interne geneeskunde II Academisch Ziekenhuis Rotterdam; R.A. Heijtink, afdeling virologie Erasmus Universiteit Rotterdam, J.A. Mazel, huisarts te Borne; M.J. Botman, Streeklaboratorium voor Pathologie en Microbiologie, Enschede; G.C. de Gast, Bloedtransfusielaboratorium Academisch Ziekenhuis Utrecht; L.J. Gerards, afdeling neonatologie Wilhelmina Kinderziekenhuis Utrecht, G.C.M.L. Christiaens, afdeling verloskunde Academisch Ziekenhuis Utrecht; J.W. Wladimiroff, afdeling gynaecologie en verloskunde Academisch Ziekenhuis Rotterdam - Dijkzigt; W.P.F. Fetter, W. Baerts, afdeling kindergeneeskunde St. Sophia Ziekenhuis, Zwolle; A.S.M. Nuijten, Rijksopleidingscentrum voor verloskundigen, Rotterdam; J. Zwijnenberg, afdeling kindergeneeskunde Ruwaard van Putten Ziekenhuis, Spijkenisse; J.R.J. Bänffer, Bacteriologisch/Epidemiologisch Laboratorium GG en GD, Rotterdam; H.Ribbers-Smeenk, Borne; H. Hofman, Utrecht; M. Bakker-Bendik, Rotterdam; J. Boot, Rotterdam; M. Voogd, Rotterdam; T.M.A. van der Laar-Kokx, Spijkenisse.

Overige mede-auteurs:

W.M. Huisman, Gynaecologie en Verloskunde Academisch Ziekenhuis Rotterdam; R.R.P. de Vries, Immunohematologie en Bloedbank Academisch Ziekenhuis Leiden.

Curriculum vitae

De auteur van dit proefschrift werd geboren op 19 juni 1964 te Rotterdam. In 1982 behaalde zij het V.W.O. diploma aan het Ichthus College te Enschede. In 1983 ging zij medicijnen studeren aan de Rijksuniversiteit Utrecht alwaar zij in 1990 het artsexamen behaalde. Hierna was zij voor een periode van een kleine 3 jaar werkzaam op de afdeling Interne Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam onder leiding van Prof. dr. S.W. Schalm. In deze periode werd gewerkt aan het onderzoek beschreven in dit proefschrift. In juli 1993 is zij in opleiding tot kinderarts in het Juliana Kinderziekenhuis te 's- Gravenhage (opleider dr. A.J. van der Heijden).

Publicaties

- Grosheide PM, Canho del R, Heijtink RA, Nuijten ASM, Zwijnenberg J, Bänffer JRJ, Wladimiroff YW, Botman MJ, Mazel JA, Gast de GC, Christiaens GCML, Gerards LJ, Fetter WPF, Baerts W and Schalm SW. Passive active immunization of infants of hepatitis B e antigen-positive mothers: comparison of the efficacy of early and delayed active immunization. Am J Dis Child: in press.

- Canho del R, Grosheide PM, Voogd-Schotanus M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of a full dose (20 μg) of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes. J Med Virol 1993; 41: 30-4.

- Canho del R, Grosheide PM, Voogd-Schotanus M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of two different dosages (10 μg and 5 μg) of recombinant DNA hepatitis B vaccine in healthy neonates. Vaccine: submitted for publication.

- Canho del R, Grosheide PM, Mazel JA, Heijtink RA, Hop WCJ, Gerards LJ, Gast de GC, Fetter WPF, Zwijnenberg J, Schalm SW and the Dutch study group Prevention Neonatal Hepatitis B. Ten year neonatal hepatitis B vaccination program, the Netherlands, 1982-1992: protective efficacy according to maternal serum levels of HBV-DNA and long-term immunogenicity. Submitted for publication.

- Canho del R, Grosheide PM, Gerards LJ, Heijtink RA, Schalm SW en de werkgroep Preventie Neonatale Hepatitis B. Praematuren en hepatitis B vaccinatie. Tijdschr Kindergeneeskd 1992; 60: 105-11.

- Canho del R, Grosheide PM, Gerards LJ, Heijtink RA, Schalm SW. Hepatitis B vaccination and preterm infants. Pediatr Inf Dis J 1993; 12: 407-8.

- Canho del R, Schalm SW, Heijtink RA. Hepatitis B revaccination of neonates with inadequate response after primovaccination. Vaccine 1992; 10: 69.

- Canho del R, Vries de RRP, Schalm SW. HLA Antigens in neonates with inadequate response after hepatitis B vaccination. Vaccine 1993; 11: 983.

- Canho del R, Grosheide PM, Schalm SW, Vries de RRP, Heijtink RA. Failure of neonatal hepatitis B vaccination: the role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates. J Hepatol: in press.

- Canho del R, Grosheide PM, Heijtink RA, Schalm SW en de werkgroep Preventie Neonatale Hepatitis B. Het beloop van hepatitis B bij kinderen die ondanks hepatitis B vaccinatie HBsAg positief werden. Ned Tijdschr Geneeskd: ter perse.

- Grosheide PM, Canho del R, Voogd M, Heijtink RA, Schalm SW and the Dutch Study group Prevention Neonatal Hepatitis B. Anti-HBs levels in infants of hepatitis B carrier mothers after delayed active immunization with recombinant vaccine concomitant with DT-polio vaccine: is there need for a second dose of HBIg? Vaccine: submitted.