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以下、 I. II. は、平成１９年度 総括研究報告書・分担研究報告書【別冊】に集録した。

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表　【書籍】

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版者名	出版地	出版年	ページ
1) Koyama T, Mito H, Takahashi K, Tanaka J, Isa K. Mushahwar, Yoshizawa H	Perinatal Hepatitis B Virus Infection in Japan	Isa K Mushahwar	Congenital and Other Related Infectious Diseases of the Newborn	Elsevier B. V.	Netherlands	2007	141-151
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IV. 研究成果の刊行物

【書　　籍】

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Perinatal Hepatitis B Virus Infection in Japan

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Introduction

In areas where infection by hepatitis B virus (HBV) is prevalent and persistent, perinatal transmission from HBV-infected mothers is an essential route for establishing a persistent carrier state.

These babies carrying HBV can transmit it via a secondary horizontal route to infants of the same generation, who frequently acquire persistent HBV infections. Approximately 30% of the infants exposed to HBV when under 3 years of age become HBV carriers. Together, they serve as a reservoir of HBV throughout their lives in the community, and may therefore cause, or contribute to, a continuous spread of the infection.

Perinatal HBV infections resulting in the persistent carrier state occur in approximately 90% of babies born to mothers who are positive for hepatitis B surface antigen (HBsAg) as well as hepatitis B e antigen (HBeAg) in the serum (Okada et al., 1976; Stevens et al., 1979). Persistent infections rarely occur in babies born to mothers who carry HBsAg, but are negative for HBeAg or are positive for the antibody to HBeAg (anti-HBe). Only around 10–15% of babies contract transient HBV infections (Shiraki et al., 1980).

In countries where the prevalence of HBsAg is less than 0.2–1.0% in children, the perinatal HBV transmission is the major route where the HBV carrier state is established. In these countries, a selective vaccination program, i.e., combined passive–active immunoprophylaxis of babies born to mothers with HBsAg and HBeAg by anti-HBs hyper-immune globulin (HBIG) and hepatitis B vaccine (HB vaccine), is a rational approach to the control of HBV infection.

In contrast, in countries where the prevalence of HBsAg exceeds 8%, perinatal transmission accounts for only 10–20% of infants who are persistently infected with HBV (Yao, 1996; Lee, 1997). Since horizontal transmission to children younger than 5 years old is the major route by which the HBV carrier state becomes established in these hyperendemic countries, universal vaccination of babies is recommended.

It is important to realize that universal vaccination prevents mainly horizontal HBV transmission, but not perinatal HBV infection. In addition, the fact that universal vaccination has the potential for inducing HBV mutants remains a serious problem of this particular approach (Zanetti et al., 1988; Brunetto et al., 1999).

In two model areas in Japan, Shizuoka and Iwate prefectures, the immunoprophylaxis of babies born to HBV carrier mothers with HBeAg, by means of combined hepatitis B HBIG and HB vaccine, was started as a clinical trial in the early 1980s and became a national project in 1986. This chapter presents an account of the experience gained in the prevention of perinatal HBV transmission from the 1980s into the 1990s in Shizuoka and Iwate prefectures.

Carrier rates of hepatitis B virus in Japan

To understand the sero-epidemiological background of HBV infection in Japan, the age-specific HBV carrier rates were estimated on a national basis (Tanaka et al., 2004). To avoid selection bias, only the data of first-time blood donors aged 16–64 years in the Japanese Red Cross Blood Center were collected and analyzed.

During the 6 years from January 1995 to December 2000, 3,485,648 individuals visited their local Japanese Red Cross Blood Centers for the first time to donate blood. The proportion of HBsAg-positive subjects, determined by reversed-passive hemagglutination (R-PHA) reagents made in-house by the Japanese Red Cross Blood Center, was calculated. To ascertain the influence of age on the proportion of HBsAg-positives, the ages of all first-time blood donors were adjusted, taking the year 2000 as the current year. The sex- and age-specific HBsAg-positive rates are shown in Table 1. Overall, HBsAg was detected in 22,018 (0.63%) of 3,485,648 blood donors. The prevalence of HBsAg was significantly higher in men (0.73%) than in women (0.53%, $p<0.001$), and increased in both with time until the age of 60 years. The HBsAg-positive rates were lowest in the age group under 20: 0.26 and 0.20% in men and women, respectively.

These data suggest that the improvement of sanitary conditions in Japan has helped to decrease the horizontal transmission of HBV and the HBV carrier state in the age groups born before the prevention of perinatal HBV transmission was started in the 1980s.

Prevention of perinatal transmission of hepatitis B virus in Japan

The prevention of perinatal HBV infections has been followed up in two model areas of Japan, namely, Shizuoka and Iwate prefectures.

Table 1

Age-specific HBsAg positive rates in first-time male and female blood donors in Japanese Red Cross Blood Center from 1995 to 2000

Age groups in 2000 (year of birth)	Total number of first-time donors	HBsAg positives (%)	Men		Women	
			Number	HBsAg positives (%)	Number	HBsAg positives (%)
16–19 (1981–1984)	582,415	1327 (0.23)	273,842	709 (0.26)	308,573	618 (0.20)
20–29 (1971–1980)	1,929,147	10,054 (0.52)	1,004,986	5955 (0.59)	924,161	4099 (0.44)
30–39 (1961–1970)	472,447	3988 (0.84)	277,627	2828 (1.02)	194,820	1160 (0.60)
40–49 (1951–1960)	247,020	2950 (1.19)	120,576	1796 (1.49)	126,444	1154 (0.91)
50–59 (1941–1950)	198,477	2984 (1.50)	80,336	1388 (1.73)	118,141	1596 (1.35)
60–69 (1931–1940)	56,142	715 (1.27)	22,782	314 (1.38)	33,360	401 (1.20)
Total	3,485,648	22,018 (0.63)	1,780,149	12,990 (0.73)	1,705,499	9028 (0.53)

In Shizuoka prefecture, immunoprophylaxis of perinatal HBV infection was initiated in 1980 as a clinical trial and became a national project in April 1986 (Noto et al., 2003). In a similar way in Iwate prefecture, following the clinical trial that began in 1981, prophylaxis of perinatal HBV infection in all babies was started in 1986 (Koyama et al., 2003).

The same protocol was used in both prefectures, and was executed as follows: babies born to HBV carrier mothers who were HBeAg reactive (high-risk babies) received HBIG at birth (within a maximum of 48 h after delivery) and the second injection was given 2 months thereafter. The babies were inoculated with HB vaccine 2, 3 and 5 months after birth and were followed until the baby reached 12 months of age (arrows, Fig. 1). In cases in which the antibody titer fell to less than 22–23 PHA, inoculation was repeated as necessary at months 9 and 12 (arrows in parentheses).

Immunoprophylaxis of perinatal HBV transmission in Shizuoka prefecture and its effectiveness in decreasing the transmission of the HBV carrier state

Shizuoka prefecture is located near the center of the main island of Japan, at the foot of Mt. Fuji, and has 3.6 million residents. In this prefecture, the first clinical trial was started in 1980 (Tanaka et al., 2004).

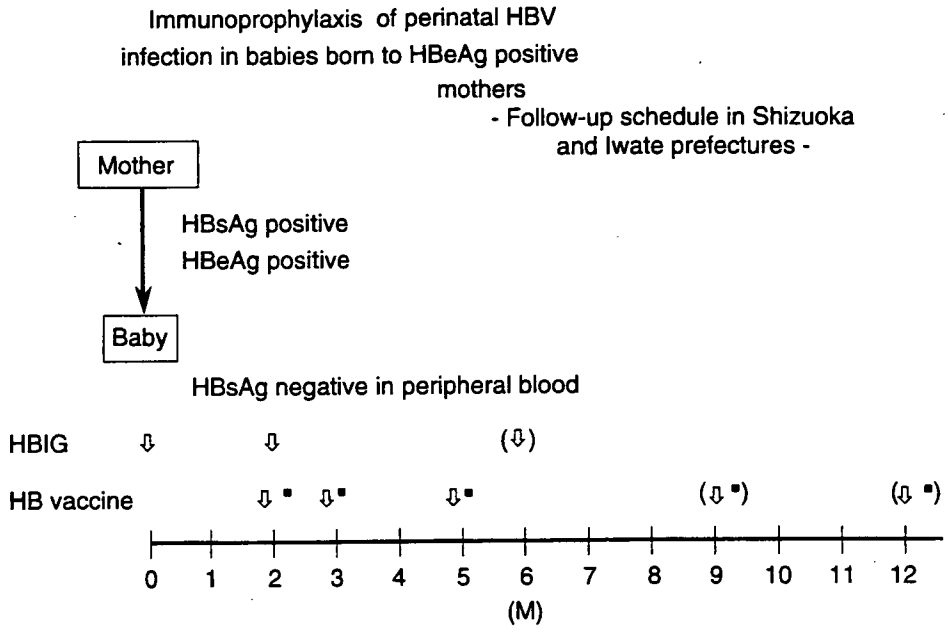

Fig. 1 Prevention of perinatal transmission of HBV: follow-up schedule in Shizuoka and Iwate prefectures. Babies born to HBsAg and HBeAg-positive mothers received two injections of HBIG ⇩ and three inoculations of HB vaccines ↓ at indicated time points. Serological tests for HBsAg and anti-HBs were performed monthly. During the follow-up period, anti-HBs titer was maintained at more than 2^3 PHA titer (equivalent to 200 mIU/ml) with appropriate use of HBIG or HB vaccine, or both, (⇩), and (↓), until 12 months after birth. During the follow-up period, when HBsAg became positive, all the prevention programs were stopped.

During the 5 years from 1980 to 1985, a total of 172 high-risk babies received immunophylaxis in a clinical trial. Of them, 166 (96.5%) were protected successfully so that they did not become HBV carriers, but this outcome could not be prevented in the remaining 6 babies (3.5%).

In 1985, the year of transition of the clinical trial to a national program status, out of 94 high-risk babies receiving immunoprophylaxis, 85 (90.4%) were protected, while the treatment failed in 9 (9.6%). During the first 9 years of the national project from 1986 to 1994, 764 high-risk babies received immunoprophylaxis. A total of 729 (95.4%) were protected, but the HBV carrier state developed in the remaining 35 (4.6%).

Overall, passive–active immunoprophylaxis following the protocol shown in Fig. 1 was effective in preventing persistent HBV infection in 980 (95.1%) of the 1030 babies born to HBeAg-positive HBV carrier mothers, but the HBV carrier state developed in the remaining 50 babies (4.9%) (Noto et al., 2003).

To estimate the efficacy of immunoprophylaxis of HBV infection, changes in the prevalence of HBsAg and anti-HBs in elementary school children (7–12 years of age) were compared in groups divided according to their birth year (Table 2). The children in group I were born before 1980 the year when the immunoprophylaxis of HBV was initiated. Those in group III were born after 1985, when the national

Table 2

Prevalence of HBsAg and anti-HBs among elementary school children in Shizuoka prefecture, divided into 3 groups according to birth year

Group	Number tested	HBsAg positives (%)	Anti-HBs positives (%)
I. Born before 1980	3446	7 (0.20)	33 (0.96)
II. Born in 1980–1985	46,993	77 (0.16)	260 (0.55)
III. Born after 1985	23,792	2 (0.01)	51 (0.21)
Total	74,231	86 (0.21)	344 (0.46)

program to prevent perinatal HBV infection commenced. The group II children were born in the period sandwiched between the clinical trial and the national program.

The prevalence of HBsAg gradually decreased from group I (0.20%), to group II (0.16%) to group III (0.01%). Likewise, the prevalence of anti-HBs decreased in a similar manner from group I (0.96%), to group II (0.55%) and group III (0.21%).

These results indicate that, in Japan, preventive measures taken against perinatal HBV infection were largely effective in decreasing the transmission of the HBV carrier state including that due to horizontal infection.

Prevention of perinatal HBV transmission in Iwate from 1981–1992 and sero-epidemiological evidence for its effectiveness

In Iwate prefecture, which has a population of 1.4 million, the clinical trial of prevention of perinatal HBV transmission was started in 1981. In 1985, the year of transition from clinical trial to national program, almost all babies born to HBeAg-positive HBV carrier mothers were given immunoprophylactic treatment (Koyama et al., 2003).

During that year, 39 (86.7%) of 45 babies who received immunoprophylaxis were protected against HBV. During the 7 years from 1986 to 1992, 100,286 (96.0%) of 104,493 pregnant women received tests for HBsAg, and it was detected in 1242 (1.2%) of them. Of those HBsAg-positives, 257 (20.7%) were positive also for HBeAg and all of their babies received immunoprophylaxis (Table 3).

The effectiveness of the immunoprophylaxis of perinatal HBV infection was clearly reflected in the changes in prevalence of HBsAg among elementary school children aged 7–12 years (Table 4). They were divided into three groups according to their birth year. In the group born between 1978 and 1980, before the start of the clinical trial of immunoprophylaxis, the prevalence of HBsAg was 0.75% (78/10,437). The prevalence of HBsAg was already decreasing among those who were born from 1981 to 1985, during the period of the clinical trial on prevention. In this group of subjects, the prevalence of HBsAg was 0.22% (46/20,812). The decrease was more prominent in children born after 1985, since the national program of

Table 3

Babies who were treated to prevent perinatal HBV transmission from 1981 to 1992 in Iwate, Japan

Year	Number of deliveries	HBsAg tested (% of deliveries)	HBsAg (+) (% of tested)	HBeAg (+) (% in HBsAg)	Prevention (% of HBeAg (+))
Before national program (1981–1985)					
1981	18,600	ND	ND	ND	1
1982	18,581	ND	ND	ND	12
1983	19,582	ND	ND	ND	18
1984	18,043	ND	ND	ND	29
1985	17,232	10,628 (61.7)	ND	45	39 (86.7%)
After start of national program (1986–1992)					
1986	16,536	15,872 (96.0)	244 (1.4)	47 (19.3)	47 (100.0)
1987	15,567	15,205 (97.7)	241 (1.6)	60 (24.9)	60 (100.0)
1988	15,410	14,282 (92.7)	166 (1.2)	40 (24.1)	40 (100.0)
1989	14,548	14,541 (99.9)	179 (1.2)	25 (14.0)	25 (100.0)
1990	14,254	13,997 (98.2)	161 (1.2)	42 (26.1)	42 (100.0)
1991	14,270	13,245 (92.8)	136 (1.0)	21 (15.4)	21 (100.0)
1992	13,908	13,144 (94.5)	115 (0.9)	22 (19.1)	22 (100.0)
Total	104,493	100,286 (96.0)	1242 (1.2)	257 (20.7)	257 (100.0)

Note: ND, no data available.

immunoprophylaxis was set in motion. HBsAg was detected in only 12 (0.04%) of 32,049 children born between 1986 and 1990 ($p<0.001$ against the prevalence in t he children born between 1981 and 1985). Likewise, the prevalence of anti-HBs decreased from 1.52% (159/10,437) in those born from 1978 to 1980, to 0.79% (165/20,812) in those born from 1981 to 1985, and 0.85% (274/32,049) in the children born between 1986 and 1990 ($p<0.001$).

The rate of anti-HBc-positives among the children with anti-HBs decreased from 81.9% (127/155) among those who were born in the years 1978–1980 to 43.3% (68/157) in those born in 1981–1985, and finally to 11.0% (59/536) in those born in 1986–1994 (Table 5). These results indicate that preventive measures against perinatal HBV infection could eventually result in the prevention of horizontal transmission among children in the same age groups.

Prevalence of surface antigen mutants

Serum HBV DNA from 15 infants and 11 mothers with chronic hepatitis acquired by either intra-uterine infection or post-vaccination prophylaxis were cloned then followed by direct sequencing of the HBV genome encoding the major antigenic

Table 4

Changes in prevalence of HBsAg and anti-HBs in 3 groups of elementary school children devided according to birth year

Year of birth	Number tested	HBsAg positives (%)	Anti-HBs positives (%)
Before immunoprophylaxis (1978–1980)			
1978	2666	26 (0.94)	52 (1.95)
1979	4212	27 (0.64)	72 (1.71)
1980	3559	25 (0.70)	35 (0.98)
Subtotal	10,437	78 (0.75)	159 (1.52)
During clinical trials of immunoprophylaxis (1981–1985)			
1981	2541	12 (0.47)	30 (1.18)
1982	1594	4 (0.25)	12 (0.75)
1983	3847	6 (0.16)	17 (0.44)
1984	6206	11 (0.18)	58 (0.93)
1985	6624	13 (0.20)	48 (0.72)
Subtotal	20,812	46 (0.22)	165 (0.79)
After start of national immunoprophylaxis program (1986–1990)			
1986	6775	3 (0.04)	41 (0.61)
1987	6505	4 (0.06)	62 (0.95)
1988	6310	2 (0.03)	58 (0.92)
1989	6436	2 (0.03)	64 (0.71)
1990	6023	1 (0.02)	67 (1.11)
Subtotal	32,049	12 (0.04)	292 (0.91)

epitopes of HBsAg (amino acids 100–200). The results of this analysis are listed in Table 6, and the following observations are listed as noted below:

1. Three novel HBV variants were detected in babies and their mothers, namely, I126T and S114T (Cases 1/I and 5/I) that were acquired by intrauterine infection. Also variant P127T in both mother and baby (Case 5/L) indicating vertical transmission. To our knowledge, these variants have not been reported previously.
2. Another novel variant, namely, G145A (Case 4/L) was detected in the baby but not his mother. This variant has been reported to occur naturally in sera of HBV chronic carriers in Korea (Song et al., 2005).
3. Analysis of all other remaining cases revealed that mixed populations of wild type and mutant viruses are found in all the tested infants and their mothers.
4. Many of the surface antigen mutants that were identified in the babies were not found in their mothers. This finding is similar to previously reported work (Nainan et al., 2002).
5. A surprising finding was the absence of supposedly the most predominant HBsAg variant, namely, G145R from the sera of the babies and their mothers.

Table 5

Prevalence of anti-HBc among elementary school children positive for anti-HBs, divided by birth year

Year of birth	Anti-HBs positive children	Anti-HBc positives (%)
Before immunoprophylaxis (1978–1980)		
1978	49	40 (81.6)
1979	72	64 (88.9)
1980	34	23 (76.7)
Subtotal	155	127 (81.9)
During clinical trials of immunoprophylaxis (1981–1985)		
1981	30	23 (76.7)
1982	12	9 (75.0)
1983	14	6 (42.9)
1984	58	18 (31.0)
1985	43	12 (27.9)
Subtotal	157	68 (43.3)
After start of national immunoprophylaxis program (1986–1994)		
1986	41	10 (24.4)
1987	61	11 (18.0)
1988	58	9 (15.5)
1989	46	6 (13.0)
1990	67	6 (9.0)
1991	62	7 (11.3)
1992	72	2 (2.8)
1993	63	5 (7.9)
1994	66	3 (4.6)
Subtotal	536	59 (11.0)

Instead, variant G145A was idetified (Cases 5/I, 2/L and 4/L). This variant has already been reported to occur naturally in sera of HBV chronic carriers in Korea (Song et al., 2005).

6. Another novel HBV variant, namely, K141E was found in a baby (Case 4/I). This is the second report of such a finding. Originally, this unique variant was identified in the sera of two Gambian children (Karthigesu et al., 1994).
7. Of interest is the presence of the novel mutant T118K (Case 2/L). This mutant has been reported once in the literature (Kfoury et al., 2001), and it is found outside the HBsAg "a" determinatnt region (amino acids 124–147) just as the case with mutant S114T (Cases 1/I and 5/I). HBsAg mutations outside the "a" determinant have been reported to damage the immunodominant region structure and thus alter the group specific dominant antigenicity (Kfoury et al., 2001). Hence, the reason we are reporting in Table 6 a variety of HBsAg mutants outside the "a" determinant itself. Current studies show that the HBsAg loop extends much further than initially believed, because other

Table 6

Mutation in the S gene of HBV DNA clones from 15 HBV positive babies and some of their mothers

Babies			Mothers		
Case/ group	Clones tested	Variant[a]	Case/ group	Clones tested	Variant[a]
1/I	21	I126T[b]	1/I	23	I126T[b]
2/I	22	Q101R, Q129R, W156R, W172R	2/I	23	P105L, T116I, K122E, C137R, L162P
3/I	6	M133T, S174G	3/I	20	A157T, L162P
4/I	12	P135S, K141E, F158S, F161S, L176P	4/I	17	Y100H, S132P, S154P, F158S, F161S
5/I	14	S114T[b], G145A[b]	5/I	16	S114T[b], G145A[b]
6/I	24	L109P, S136P, S155Y	6/I	23	T113A, G119R, S136P, T148A
7/I	22	S113A, S117C, T118P, C139Y N146D, L176P	7/I	24	G112E, S117N, Q129H, S132L, C149Y, R160G, F170L
8/I	23	P142L	8/I	23	L109P, S114P, C124R, T131A, C138R, S171F, W172C, W172R
9/I	24	G130E, T148A, C149R, S171F	9/I	24	S117R, C121G, Q129P, M133I, P135L, S171P
10/I	17	C121S, T115A	10/I	0	NT
1/L	10	T116A[b]	1/L	0	NT
2/L	10	T118K, G145A	2/L	0	NT
3/L	10	I126T, T131A	3/L	0	NT
4/L	16	G145A[b]	4/L	14	C124R, C137R
5/L	19	P127T[b]	5/L	20	P127T[b]

Note: I, intrauterine infection; L, late-phase infection; NT, Not Tested.

[a]Amino acid substitution in "a" determinant (124–147) and outside "a" determinant (100–123 and 148–176).

[b]Novel variants, no wild-type virus present.

conserved epitopes have been found also between amino acids 100 and 200 (Kfoury et al., 2001; Gerlich, 2004).

The data shown in Table 6 illustrate that changes in either the major hydrophilic loop of HBsAg or outside it are common and do play an important role in transfusion safety, HBV vaccine efficacy and diagnostic accuracy and reliability.