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Viral Hepatitis and Hepatocellular Carcinoma Prevention Strategy in Japan

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The study of human hepatitis, particularly in Asia, where the incidence rate has been the highest in the world, has contributed greatly to the understanding of carcinogenesis of the liver and related diseases. In this article, the history of research on hepatitis viruses and hepatocellular carcinoma (HCC) and the successful prevention of vertical transmission of hepatitis B virus (HBV) in Japan are reviewed, focusing on the studies that resulted in the identification of vertical transmission of HBV infection and the association of HBV-sustained infection and HCC. The vaccination trials for preventing HBV vertical transmission and the fruitful outcome of the nationwide vaccination strategy in Japan, on the basis of "selective" immunization by using anti-HBs immunoglobulin (HBIG) and HB vaccine, are highlighted. Ongoing studies on the mechanisms underlying hepatocarcinogenesis induced by viruses, e.g., the roles of viral proteins and inflammation, are also reviewed, and prospects for the control of HCC are discussed.

Key words: Hepatocellular carcinoma — Hepatitis B virus — HB vaccine — Vaccination strategy

Miura first recorded primary liver cancer in Japan in 1889, as cited by Yamagiwa in his article of 1911.[1] Most of the papers that had appeared in Europe and the United States up to that time may have described metastatic liver cancers, as the two types of liver cancers were not distinguished. Yamagiwa named the two types of primary liver cancer "hepatoma" and "cholangioma."[1] In 1914, Nagayo classified liver cirrhosis into types A and B,[2] and Kika claimed an association of liver cancer with type B liver cirrhosis in 1927.[3] The high incidence of liver cirrhosis and liver cancer in Japan was supposed to be attributable to certain environmental factors peculiar to Japan. Many physicians and scientists in Japan tried to identify the causative agents, and clues to the role of hepatitis viruses as etiological agents came from epidemiological and clinical studies.

Another reason for the progress of hepatocellular carcinoma (HCC) research in Japan is that the nation became the center of studies on experimental chemical carcinogenesis in the liver. In 1915, Yamagiwa and Ichikawa released their study on coal tar-induced cancer in rabbit ear.[4, 5] In 1932, Sasaki and Yoshida described the use of *ortho*-aminoazotoluol from the viewpoint of its specific affinity for the liver: azo pigments proved to be good liver carcinogens.[6, 7] Later, aflatoxin[8, 9] and heterocyclic amine[10, 11] were proved to be hepatocarcinogens, and many studies have been conducted on their action. From this historical background, scientists and physicians worldwide, especially those in Japan, have sought to understand and to control viral hepatitis and hepatocellular carcinoma.

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The Search for a Virus Inducing Hepatocellular Carcinoma (HCC)

Initial stage of research on hepatitis viruses

The indication that HCC is of viral origin derived from epidemiological studies. The study on liver diseases started at the same time as the search for the cause of jaundice and liver cirrhosis, and these diseases were found to be infectious. Amano suggested in 1952 that viral hepatitis might develop into cirrhosis followed by cancer, a great insight for those early days.[12] As has always been the case, phenomena are looked at first, and in this case the cause of HCC as well as hepatitis was considered to be associated with jaundice and liver cirrhosis.

It was through a report by Hiro and Tasaka,[13] that we first learned that hepatitis is caused by viruses. His paper presented a successful transmission of jaundice to human volunteers by coating the pharyngeal mucous membranes with ultrafiltrate of the sera from other patients with acute hepatitis. Voegt[14] and MacCallum and Bauer[15, 16] obtained similar results by the oral administration of duodenal juice or by the parenteral administration of hepatitis serum.

Perinatal transmission of HBV and HCC

The discovery of Australia Ag (Au-Ag, HBsAg) by Blumberg in 1964,[17, 18] and the clarification of its significance as an antigen related to post-transfusion hepatitis by Okochi *et al*. in 1970,[19] made it possible to pursue the

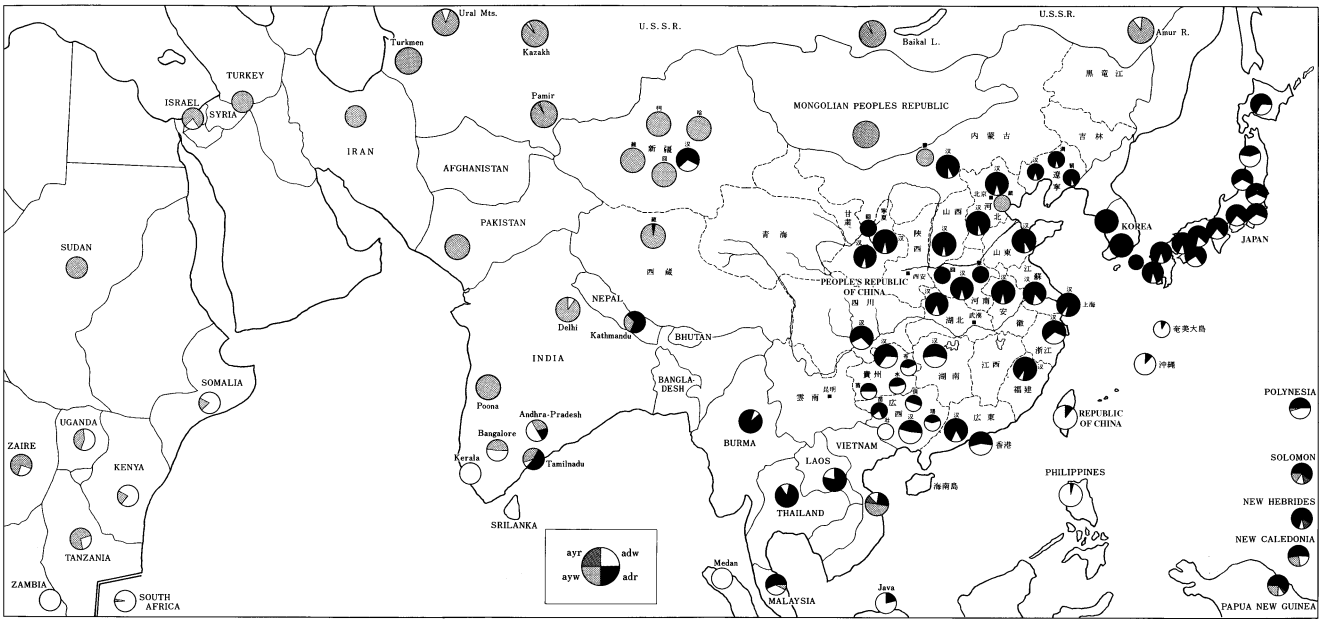

Fig. 1. Distribution of HBV subtypes. The figure is from reference 21) with a slight modification.

hepatitis viruses. The first nationwide study of HBsAg prevalence and distribution in Japan was made by our study group[20]; the positivity rate was 2.6% of the population in 1973. The worldwide number of hepatitis B virus (HBV) carriers is estimated to be 280 million, and 78% of them are living in Asian countries. There are four major subtypes of the virus: ayw, adr, adw and ayr, which are coded by the virus genome. Therefore, to elucidate the modes of transmission of this virus, the distribution of these subtypes has been investigated. In three different zones of Asia, a characteristic subtype predominates, as shown in Fig. 1.[21] The zone for adr is from northeast Asia (Japan, Korea, the Han ethnic group in mainland China) to southern Asia (Laos, Thailand and Malaysia) and includes agrarian areas that are heavily forested. The Tamil region and Nepal form the borderline between the adr and ayw zones.[21] Zone adw encompasses the southernmost parts of Asia, such as Okinawa, Taiwan, the Philippines, Indonesia, minorities in southern China, the southwest coast of India and the east coast of the African continent, crossing over the Indian Ocean and Pacific Ocean to the Americas. The zone for ayw is mainly western Asia (the USSR, north India, Iran, Israel), extending to the Mediterranean area and to northern and central Africa and over steppe or desert areas.

In 1972, Obayashi, Okochi and Mayumi reported the familial clustering of HBsAg and demonstrated mother-to-child transmission of HBV in Japan.[22] During the course of their study, they found HBsAg in the sera of 20 out of 24 (83%) children of HBsAg-positive female siblings, whereas only 1 of 7 children of HBsAg-positive male siblings was antigen-positive. Anti-HBs was found in this child's mother. The subtype of HBsAg within a family was always identical. This was the first observation supporting mother-to-child transmission of HBV infection. In such families, some members developed chronic hepatitis, liver cirrhosis or liver cancer. In 1975, maternal transmission of HBV infection to infants born to asymptomatic carrier mothers was reported in Tokyo and in Taiwan. Since then, many cases of perinatal transmission of infection from HBV carrier mothers to their babies have been observed, resulting in the identification of an HBV carrier state that may be a critical etiological factor in persistent HBV infection and HCC. The risk of infection varies from country to country. In general, it is higher for babies born to carrier mothers in countries with high carrier rates, especially in Asian countries or Asian ethnic groups, than for similar babies in countries with low carrier rates, e.g., those in Caucasian ethnic groups. The prevalence data in Table I[23] and Table II[31] show the mode and magnitude of perinatal transmission in each region in the world. These data provide prevalence rates useful to develop strategies for prevention of HBV.

HBV as a cause of HCC

HCC often accompanies liver cirrhosis (LC), but the frequency of this combination varies greatly from place to place. Finding an explanation for this fact is one of the most important purposes of research on the causes of LC. Alcohol poisoning and chemical or bacterial toxins were

Table I. Perinatal Transmission Pattern of HBV in Various Countries with Regard to HBe Antigen/Antibody Status[23)]

	$HBeAg^+/HBsAg^+$ rate in pregnancies (%)	Follow up cases of HBs mothers		No. of HBs babies (%)
		HBe status	No. of mothers	
Burma	296/792 (37.3)			
China (1980–82)	22/276 (26.1)	$HBeAg^+$	56	48 (85.7)
		$HBeAb^+$	48	12 (25.0)
Hong Kong	38/78 (54.3)	$HBeAg^+$	22	22 (100)
		$HBeAg^-$	13	5 (38.5)
Japan (1976–82)	341/1296 (26.3)	$HBeAg^+$	200	170 (85.0)
		HBeAg, Ab^-	274	6 (2.2)
		$HBeAb^+$	353	0 (0)
Philippines (1982)		$HBeAg^+$	10	6 (60.0)
		$HBeAb^+$	51	1 (1.9)
Singapore (1982)		Chinese	58	26 (44.8)
		Other races	58	17 (29.3)
Taiwan (1977)	20/62 (32.3)	$HBeAg^+$	20	17 (85.0)
		$HBeAg^-$	42	13 (30.9)
Thailand (1978–80)	14/42 (33.3)	$HBeAg^+$	14	12 (85.7)
		$HBeAg^-$	28	0 (0)

Table II. Patterns of Hepatitis B Prevalence[a), 31)]

	Low	Intermediate	High
HBsAg	0.2–0.5%	2–7%	8–20%
Anti-HBs	4–6%	20–55%	70–95%
Childhood infection	Infrequent	Frequent	Highly frequent
Neonatal infection		Frequent	Highly frequent
Location	Australia	Eastern Europe, Japan	Some parts of China
	Central Europe	Mediterranean	Southern Asia
	North America	South-West Asia, USSR	Tropical Africa

a) Prevalences up to 50% have been identified in some isolated Pacific islands.

regarded as the main causes. In Europe and the United States, LC does not accompany HCC in general, whereas in Japan and Asian countries LC often accompanies HCC. As alcohol was considered to be mainly responsible for the development of LC in Europe and the United States, the LC seen in those two areas and that in Japan were considered to be different. This inference met with strong opposition from many researchers throughout the world when presented at the Congress of Geological Pathology in Northern Europe in 1952. As previously mentioned, Amano's assumption was that the virus itself might be the cause in Japan, because of the difference in pathological features of cirrhosis in Europe and in Japan. That is, the cirrhosis in Europe might be mainly caused by alcohol consumption, while that of our nation might be mainly caused by virus infections.

There were only a few case reports at that time from Europe and the United States on patients with both viral hepatitis and HCC, including those by Sheldon and James,[24)] and Walsche and Wolffe.[25)] In Japan, a long-term follow-up study of viral hepatitis conducted by Kosaka *et al*. revealed some cases of HCC. But the types of hepatitis viruses in those cases have never been identified. Statistics of the National Cancer Center of Japan (Arima, 1968), showed that of 238 cases of hepatitis accompanied by cirrhosis, 39 cases had HCC, and of these 39 cases, 16 had had transfusion and 21 had hepatitis patients within their families. These facts gradually drew the attention of investigators around the world. At that stage of the research, Au-Ag or HBsAg, was discovered. The problem of hepatitis viruses was highlighted in the hepatitis symposium for the WHO report on viral hepatitis,[26)] especially in Nishioka's seroepidemiological work[27–29)] and Obayashi's work[22)] concerning LC and families with Au-Ag. A 1975 WHO report also noted that HBsAg was very often detected in the serum of HCC patients in Africa, Japan,

Table III. Recommendations for Hepatitis B Vaccine Prophylaxis in Relation to Prevalence of HBV[31)]

Low prevalence		Intermediate or high prevalence	
Pre-exposure	Post-exposure	Pre-exposure[a)]	Post-exposure
High risk groups (health care personnel, dialysis patients, institutionalized patients, drug addicts, male homosexuals, military recruits)	Accidental percutaneous exposure, infants of HBsAg-positive mothers, sexual contacts of acute cases, and carriers	All infants or selected	Infants of HBsAg-positive mothers

a) On the basis of "selected" in Japan.

and other parts of Asia, where there were many cases with HCC. Shikata *et al*.[30)] demonstrated the presence of HBsAg inclusion bodies by orcein staining.

The WHO guidance for a vaccination strategy was taken up in a task force meeting held in Nagasaki in 1985[31)] (Table III). It was noted that in Africa and the Asia-Pacific region, HBV carriers accounted for 5–20% of the whole population. In these regions, the incidence of HCC is also high, with a maximum incidence of 150 cases per 100,000. HBsAg was detected in 50–80% of these HCC patients, and they are HBV carriers. In contrast, there are only a small number of HBV carriers in North America and Europe: only 0.13–1.0% of the population. Also, the incidence of HCC is very low, with 1–3 cases per 100,000. In these areas, 5–30% of HCC patients are HBV carriers. In the United States and Europe, the main cause of LC is alcoholic liver damage, and in both regions the numbers of HBV carriers are small. Except in cases accompanied by alcoholic cirrhosis, the positivity rate of HBsAg in HCC is still high; namely, it is on the same level as in regions where there are many HCC patients. Among other investigations of HCC in Africa, Asia and other parts of the world, one report stated that of a total of 2,387 cases studied, there were 1,439 cases (53.6%) in which HBs antigen was detected, while for the 20,251 cases in the control group, there were 823 (4.1%) in which the antigen was detected. The region that showed the highest percentage, 71.9%, was the Pacific region, while the value in the control group was 0.3%.[32)] We can say that in regions with many HBV carriers, large numbers of HCC cases are reported, and the ratio of HBV carriers relative to HCC patients is high. Therefore, it is almost certain that there is an etiological relationship between sustained HBV infection and HCC. In Japan, an investigation was conducted by the Liver Cancer Study Group of Japan[33)] for a two-year period, 1980–1981, covering 405 facilities throughout the country. The results showed that of 1,645 cases of HCC, HBsAg was detected in 517 cases (31.4%). According to the clinical tracking report, the most dangerous infection occurred in the neonatal period, which most probably induces HBV carrier status.

Prevention of Perinatal HBV Transmission

Selective immunization, combined passive and active immunization

Successful prevention of perinatal transmission of carrier state with human anti-HBs immunoglobulin (HBIG) and HBV vaccine was first reported by Tada *et al*.[34)] We favored a selective immunization strategy in place of universal immunization. Follow-up studies of the infants born to HBeAg-positive carrier mothers showed that 80 to 90% of these infants had persistent HBsAg. In Japan, approximately 2 to 3% of pregnant women are HBsAg carriers; 20 to 30% of them are also positive for HBeAg.[35)] In Taiwan, 15 to 20% of pregnant women are HBsAg carriers; 40% of these women are HBeAg-positive.[36)] In contrast, infants born to anti-HBe-positive chronic carrier mothers rarely become HBsAg carriers, although they may develop HBsAg transiently in association with an elevation of serum amino transferase levels at between 2 and 4 months of age.[37)] Based upon these results, efforts to prevent vertical transmission of HBV in Japan have been directed largely toward infants born to HBeAg-positive carrier mothers. Members of the Study Group for the Prevention of Vertical Transmission of HBV carried out a study, which involved more than 30 hospitals throughout Japan.[38)] Pregnant women attending obstetric clinics were screened for HBsAg by reverse passive hemagglutination (RPHA) or radioimmunoassay (RIA), and for HBeAg and anti-HBe by RIA. Pregnant women positive for anti-HBe and their infants were also followed but were not given immunoprophylaxis. At delivery, the cord blood was tested for HBsAg by RPHA and positive infants were not given immunoprophylaxis, because of *in utero* infection, but babies whose cord blood was positive for HBsAg only by RIA were included in this study.

Efficacy trial of HBIG and hepatitis B vaccine for the prevention of perinatal HBV transmission

Before the HBV vaccine became available for clinical trials, we were already using HBIG for prevention of the mother-to-infant transmission of HBV. These procedures

were started in 1977 in Japan.[39, 40] HBIG was given intramuscularly immediately after birth to neonates born to HBeAg-positive carrier mothers and repeated injections were given every 3 months until the child reached 1 year of age. Therefore, when the HBV vaccine became available in 1980, some of the infants had already received HBIG. Those children were given HBV vaccine (10 μg) three times, starting at 6 months of age or later (protocol 2, Fig. 2). However, since the vaccine became available, HBIG and vaccine treatment have been given in another regimen. Here, the administration of HBV vaccine (10-μg dose three times), was started at the age of 2 to 3 months after birth, following two injections of HBIG (protocol 1, Fig. 2).

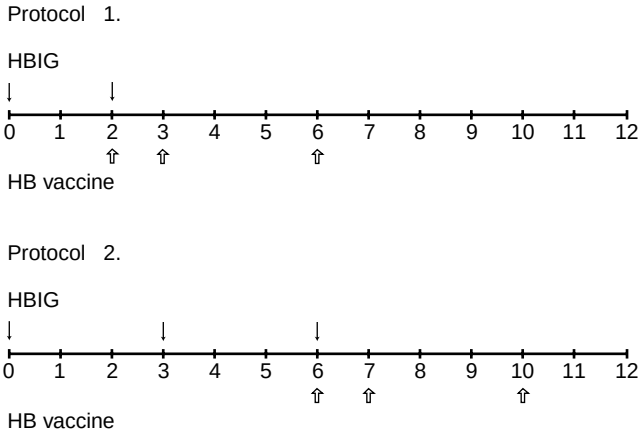

Fig. 2. Schedule of administration of HBIG and HBV vaccine for prevention of the vertical transmission of HBV. In Protocol 1, HBV vaccination was started within 3 months of birth and in Protocol 2, it was started at 6 months after birth or later.[43]

For HBV vaccine, HBsAg (subtype adr) was purified by Takahashi in the Kitasato Institute. A dose of 1 ml of precipitated HBV vaccine contained 40 μg of HBsAg protein and 0.25 mg of aluminum hydroxide. These vaccines were prepared from plasma of HBeAg-negative chronic HBsAg carriers and were inactivated by exposure to both heat and formalin.[41, 42] Each vaccine dose consisted of 10 μg and each child received three injections. Vertical transmission of HBV was prevented in 848 of 881 infants (96%): 33 infants (3.7%) became chronic HBsAg carriers despite the prophylaxis. However, in studies of infants born to HBeAg-positive carrier mothers before the availability of preventive measures, 130 of the neonates (89%) developed the chronic HBsAg carrier state (Table IV).[43] In contrast, none of the 262 babies born to anti-HBe positive carrier mothers became HBV carriers, but 10 cases (4%) developed HBsAg transiently with elevation of serum aminotransferase levels between 2 to 4 months of age (Table V).[43] The optimum strategy of application of the HBV vaccine may differ from country to country. There are two main reasons for the use of the HBV vaccine. The first is to prevent liver cirrhosis and hepatocellular carcinoma related to persistent infection with HBV. The second is that it is important to administer the vaccine early in life. Application of the vaccine to infants born to noncarrier mothers is a straight-forward approach to this problem. On the other hand, one year follow-up studies of 583 infants born to HBeAg-positive mothers and treated by protocol 2, starting vaccination between 2–4 months, were reported by Matsumoto, Takeuchi, Yano, Shiraki and Kawana in the Collaborative Research Group. They showed that 21

Table IV. Efficacy of HBIG and HBV Vaccine for Preventing Vertical Transmission of HBV[43]

Treatment regimen	No. treated	No. prevented	No. developing HBsAg
Protocol 1	617		24 (4%)
Protocol 2	264		9 (3.4%)
Both protocols	881	848 (96.3%)	33 (3.7%)
Before vaccine	146		(84.9%), 124 carriers

Table V. Course of Infants Born to Chronic HBsAg Carrier Mothers Who Were not Given Immunoprophylaxis[43]

Mother's HBeAg/Anti-HBe status	No.	No. (%) of infants developing	
		Transient HBsAg	Chronic HBsAg carrier state
HBeAg	146	6 (4)	124 (85)
Anti-HBe	262	10 (4)	0 (0)

infants became HBV carriers and the protective efficacy rate was 95.7%. Shimizu reported that in collaborative studies with 43 institutions employing protocol 1, a 97.6% protective efficacy rate was obtained, i.e., 688 infants out of 702 did not show HBs antigenemia.[44] Our National Research Group summarized the results obtained by each group (Table VI).[38] Among the studies conducted, the one year follow-up studies of infants born to HBeAg-positive mothers and treated by protocol 1, which constituted the main body of our collaborative work, showed a 93.7% protective efficacy rate (infection rate 6.3%). The rate of acquisition of carrier status was only 3.6%, which differed greatly from the value of 92.5% of historical control babies before vaccine availability. We chose not to conduct a randomized control trial using newborn babies, because the protective efficacy rate was so high. In our trial, however, 33 infants (3.7%) developed persistent HBV infection despite HBIG and HBV vaccine treatment as stated earlier. The 22 infants who became HBsAg-positive within 6 months after birth might have been infected *in utero*. The 11 infants who became positive at 6 to 12 months of age can be regarded as nonresponders to the HBV vaccine. These low responders to HBV vaccine should be considered candidates for further HBIG or vaccine administration. Preventive procedures using HBIG and HBV vaccine against vertical transmission are clearly effective and may provide a means to reduce greatly the incidence of liver cirrhosis and hepatocellular carcinoma in areas of the world with a high prevalence of HBV infections.

Nationwide vaccination strategy for preventing HBV vertical transmission and its fruitful outcome in Japan

In Japan, since January 1, 1986, initiatives have been taken to prevent mother-to-child transmission of HBV. Babies born to mothers positive for HBe antigens are treated by selected immunization procedures, financed by the central and local governments. About 4,500 newborn babies a year receive 1,000 IU of HBs antibody γ-globulin. This is followed with HBV vaccine (HBsAg 10 μg) 2 months after birth, plus additional vaccine treatment 3 months and 5 months after birth. The assumption was that this treatment would prevent about 95% of the vertical transmission. Indeed, HBV carrier babies were successfully decreased to about 520 a year, in 1989 (Table VII).[45] HBsAg screening of donated blood is now almost complete (Table VIII),[46] and the prevalence of HBsAg should steadily decline (Table IX).[46] If this trend continues, it is expected that hepatitis B will be eradicated in Japan in about 50 years. We are aware that improved vaccine, especially that containing HBV pre-S region, is much more effective. Since 1986, HBV vaccine has also been administered to babies born to HBsAg-positive mothers without HBeAg. The former Director of WHO, Dr. Mahler, emphasizes that the HB vaccine trials are the first ever to focus on an immunological measure for the prevention of human cancer. Recently, however, Marshall[47] raised the possibility that recombinant vaccine (HBsAg) may provoke an autoimmune attack on a similar protein in the nerves or other tissues of a genetically susceptible group of vaccine recipients, because of molecular mimicry. If that is so, vaccine recipients may have to be restricted. Our strategy of selective immunization might be appropriate.

Hepatitis C Virus as a Cause of HCC

Although in Japan HCC is one of the most prevalent cancers, at most, 25% of HCC patients are positive for HBsAg. Thus, another virus may be involved in liver carcinogenesis. A nationwide surveillance study of Non A Non B hepatitis was also made by our group.[48, 49] A potential role of hepatitis C virus (HCV) in the development of HCC was revealed by an HCV antibody assay in 1988.

Table VI. Effect of HBIG, HBV Vaccine Treatment

Treatment		No. of cases	Becoming carrier	Transient infection	Infection rate
Before Vaccine		206	173 (84.0)	>23 (>11.2)	>92.5
HBIG i.m.	Once	49	22 (44.9)	13 (26.5)	71.4
	Repeated	200	38 (19.0)	>51 (>25.5)	>44.5
HBIG i.m.	5– mo.	112	9 (8.0)	6 (5.4)	13.4
+after birth Vaccine[a]	2–4 mo.	583	21 (3.6)	>16 (2.7)	> 6.3
	0–2 mo.	16	1 (6.3)	0 (0)	6.3
HBIG i.m., i.v.[b]					
+after birth Vaccine[a]	2–3 mo.	702	14 (2.0)	6/307 (2.0)	4.0

( ): %. Summarized by Collaborative National Research Group on Hepatitis Prevention,[38] chaired by Iino and Oda, 1985.
a) Vaccination, starting at 5–, 2–4, 0–2, 2–3 mo. after birth 3 times, respectively.
b) HBIG i.v., pepsin-digested HBIG.

Table VII. Nationwide Strategy for Preventing HBV Perinatal Transmission and Its Successful Outcome[45]

HBsAg of all pregnant women was tested, since Oct. 1985.
HBIG and vaccine administration using funds from government, central and local, since Jan. 1, 1986.
— HBIG 200 IU at delivery and after 2 months, then HB vaccine 10 μg s.c., 2, 3, 5 months, three times after birth.
Outcome (Fall, 1985–March, 1991):
Total number of pregnant women tested: 6,489,005 (94.0–96.8% of all pregnant)
Number of babies treated with HBIG and vaccine: 19,247
HB carrier babies:
Before treatment, 4,500 per year
In 1990–1992, 404 per year (0.03% of newborn babies)

By Shiraki, K. (Chairman of Nationwide Surveillance Group).

Table VIII. Reduction of Post-transfusion Hepatitis[46]

Screening donated blood		No. of cases	Post-transfusion hepatitis (%)
HBsAg	1988–1989	1,581	4 (0.25)
HBsAg+HBcAb	Oct.1989–1990	908	0 (0)

Red Cross Blood Center.

Table IX. Reduction of HBsAg Positivity Rate in Japan[46]

HBsAg positivity	1973	2.6%[a]	(IAHA method)
Rate	1991	0.92%	(RPHA method)

a) Of the population.

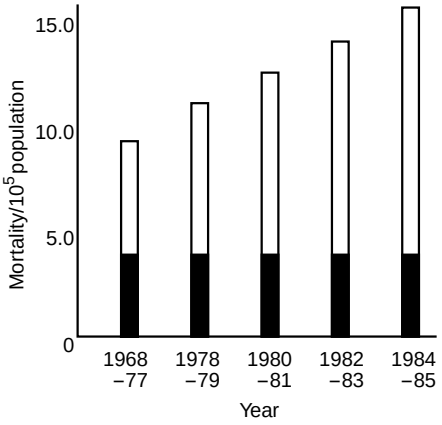

Fig. 3. Estimation of HBsAg-positive (■) and HBsAg-negative (□) liver cancer based on the mortality rate and HBsAg positivity for hepatocellular carcinoma in Japan.[50]

Sera from 105 HBsAg-negative HCC patients were collected and assayed for antibodies to HCV antigen (HCVAb). Most of these patients (76.2%) were found to be positive for HCVAb, even though the prevalence in sera from blood donors was 1.1%. A history of blood transfusion was confirmed in 39.6% of the cases positive for HCVAb, which was significantly different from the lower rate (4.7%) observed in HCC patients with both positivity for HBsAg and negativity for HCVAb (P<0.001). Statistics in Japan indicate that the numbers of fatal cases of liver cancer reported to the Ministry of Health and Welfare of Japan have dramatically increased from 1978 to 1985, as compared with those from 1968 to 1977. The number of deaths due to liver cancer each year per 10^5 was 9.5 in 1968 to 1977, and 16.0 in 1984 to 1985, as shown in Fig. 3.[50] However, the numbers of the HBsAg-positives among the HCC patients have decreased from 1968 to 1985, according to the reports by the Liver Cancer Study Group of Japan: 40.7% (1968–1977), down to 24.6% (1984–1985). Based on these figures, it is estimated that the mortality rate in association with liver cancer and HBsAg positivity is 3.9 (1968–1977) and 4.0 (1984–1985) per year per 10^5.

Mechanisms of Viral Hepatocarcinogenesis and Control of Hepatocellular Carcinoma

The relationship between hepatitis and HBV has been established through the identification of HBV-DNA integration into human hepatocyte DNA,[51, 52] and we suspected that the HCV virus also plays an important role. The cloning of human viruses triggered a rush of studies on carcinogenic mechanisms at the gene level. We now need close cooperation between basic research and the clinical side to track such cases over time. Further, as the X gene of HBV (*HBx*) has been shown to be a transactivator, transgenic mice carrying *HBx* gene were generated[53] to test the hypothesis that the viral transactivator may alter host gene expression and lead to the development of

hepatic cancer. Recently, HCV core protein has also produced HCC,[54] and induced multifocal histopathological changes in the liver, beginning with foci of vacuolated altered hepatocytes with fatty liver around central veins, followed by benign adenoma and progression through multistep alterations to malignant HCC. Chemical carcinogenesis and viral carcinogenesis are most likely to be linked in some way.

Prevention of Hepatitis Virus Infection and Liver Cancer

Primary prevention is the avoidance of exposure to the viruses by improving hygienic conditions and taking proper medical precautions, including screening of blood for transfusion and single use of needles, and the enhancement of immunological competence against primary exposure to the viruses by nonspecific measures such as improvement in nutrition or immunomodulators, and by specific measures such as HBIG and HBV vaccine. *Secondary prevention* includes early-stage diagnosis by ultrasonography, computed tomography, or tumor markers followed by noninvasive or surgical treatment. To intervene in *progressive inflammation*, that is, to interfere with the disease progression from persistent viral infection to its sequelae, antiviral agents, interferon or immunomodulators are available for patients with HBV, and especially HCV persistent infection. Recently, lamivudine and rivavirin have been introduced. Antiinflammatory agents such as glycyrrihizin or sho-saikoto have also been applied in persistent chronic hepatitis, and decreased ALT and AST levels to some extent. Randomized controlled studies[55–57] revealed that these antiinflammatory procedures showed a small but significant prolonging effect on the hepatocarcinogenic process.

Conclusion and Prospects

The study of human hepatitis, particularly in Asia, where the incidence rate has been the highest in the world, has contributed greatly to the understanding of carcinogenesis of the liver and related diseases. Of hepatitis cases, about 10% are hepatitis B and the remaining 90%, HCV and others. As many as nearly half of all chronic liver disorders are thought to be caused by HCV, which has markedly increased in the United States, partly due to illegal drug use. The prevention of HBV mother-to-child transmission remains one of the most important ways to prevent the occurrence of viral hepatitis and HCC as well, and similar considerations apply to HCV. In Japan, selective immunization has reduced the number of HBV carrier-babies to about 200 a year. In addition, following the addition of an HBc antibody check to the original HBsAg one for screening donor blood, post-transfusion hepatitis due to HBV had completely vanished in Japan in 1991.[45] The HBsAg positivity rate was reduced to 0.92% of the population in 1991, although it was 2.6% in 1973.

It is, of course, very important to know how the host human body reacts to viral attack. As with all bacterial and viral infections, the host reaction is one of the key factors in the progress of infectious diseases, in addition to the character of the attacking organism. In other words, it is necessary to understand the mechanisms of viral carrier status and carcinogenesis, in order to provide a rational basis for prevention and treatment.

As with other cancers such as colorectal carcinoma, a multistep process is presumably involved in the development of HCCs, a majority of which is known to be associated with viral hepatitis B or C. Multiple factors play roles in such stages as the initiation of normal cells, promotion of pre-neoplastic cells, recruitment of vessels that feed malignant cells or the escape of cells from immune surveillance. Hepatitis viruses themselves, both HBV and HCV, assume a critical role in the transformation of hepatocytes through their encoded proteins, the HBx and the HCV core proteins, respectively. Along with the viral products, hepatitis itself may promote hepatocarcinogenesis: inflammation, which is induced by viruses and mediated through immune reaction, causes continuous cell death and regeneration, resulting in an increase of mutations in the host genome. The accumulation of genetic aberrations giving a growth advantage to the cells may eventually lead to the development of HCC. In the case of HBV infection, mutations in the core promoter are closely associated with an active phase of chronic hepatitis, and may be involved in hepatocarcinogensis by prolonging the duration of active hepatitis. They may perform a role in clearing HBeAg (Yano, M., personal communication).

Chronic viral hepatitis, particularly in its advanced stage, corresponds to the "*hypercarcinogenic state*," which is related to *inflammation-mediated carcinogenesis* as designated by Hino *et al*.[58] Efforts to convert the *hypercarcinogenic state* to a *normo- or hypocarcinogenic state* must therefore be made to block or modulate carcinogenic activities. Vaccination, antiviral agents, immunomodulators or anti-inflammatory agents may modify the activities of the viruses or hepatitis and derail the process of hepatocarcinogenesis. New techniques such as *missile therapy* and *human monoclonal antibodies* have great potential. We cherish the hope that persistent effort will result, ultimately, in the prevention of cancer.

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