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# Prevention of liver cancer

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Report of a  
WHO Meeting

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## WHO MEETING ON PREVENTION OF LIVER CANCER

Geneva, 30 January–4 February 1983

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# PREVENTION OF LIVER CANCER

## Report of a WHO Meeting

A WHO Meeting on Prevention of Liver Cancer was convened in Geneva from 30 January to 4 February 1983. The meeting was opened by Dr H. Mahler, Director-General of WHO, who noted that there were unique opportunities now, for the first time, to prevent one form of cancer by immunization. Results from basic research and field studies suggested that the time was ripe for research on strategies to prevent hepatitis B infection, chronic liver disease, and liver cancer. Chronic liver diseases are important, especially in the developing countries, and Dr Mahler indicated that consideration was being given by the World Health Organization to the establishment of a global hepatitis programme. He pointed out that liver cancer was one of the ten most prevalent cancers in the world, and one of the most frequent cancers in developing countries.

An effective hepatitis B vaccine suitable for global immunization could have a considerable impact on health. Dr Mahler asked the Meeting to review the evidence that immunization could prevent the chronic carrier state of hepatitis B virus infection and that this would lead to a decrease in liver cancer.

## 1. EPIDEMIOLOGY OF LIVER CANCER

### 1.1 Descriptive epidemiology

Primary liver cancer includes hepatocellular carcinoma (relatively common), cholangiocarcinoma (rare), angiosarcoma (very rare), and other even rarer forms of cancer. These entities are not only pathologically distinct but also etiologically different. However, since hepatocellular carcinoma is by far the most common of these cancers in virtually every country of the world, the descriptive epidemiology of primary liver cancer is very similar to that of hepatocellular carcinoma.

Primary liver cancer is among the most common cancers in WHO's South-East Asia and Western Pacific Regions, and perhaps

the most common of all cancers in Africa south of the Sahara (particularly in the south-east). Since many of these areas are heavily populated, primary liver cancer is among the ten most common cancers in the world, and it is estimated that there are at least 250 000 new patients with liver cancer each year. Estimation of the frequency and study of the descriptive epidemiology of such cancers are hampered by the difficulties involved in the definitive diagnosis and correct classification of the disease. For example, mortality figures are distorted by misclassification of other cancers that present with metastases in the liver, incidence data are limited, and autopsy series are difficult to interpret and are substantially affected by selection.

From the published studies, it appears that the proportions of primary liver cancer among all cancers and among all autopsies are 30% and 5%, respectively, in high-incidence countries, 10% and 2% in intermediate-incidence countries, and 2% and 0.5% in low-incidence countries. The actual incidence of the disease is at least 30 new cases per 100 000 population each year in some parts of Asia and Africa, whereas it is less than 5 new cases per 100 000 population per year in Australia and in most populations of Europe and North America. The incidence appears to be increasing with time in the majority of the low-incidence countries.

Primary liver cancer is more common among males than females, the male:female ratio being higher in the autopsy-diagnosed cases (median, around 5) than in the reported mortality rates (median, around 2). This difference is again probably due to the confusion with other cancers that present with liver metastases. It is well established that the incidence of primary liver cancer increases with age, but in high-risk populations it also occurs in younger age groups. The disease shows a marked increased incidence in certain ethnic groups.

Since this Meeting was concerned with the practical issues involved in the prevention of liver cancer, attention was focused only on the overwhelmingly more frequent hepatocellular carcinoma, particularly in view of its now well defined viral etiology.

## **1.2 Etiology of hepatocellular carcinoma**

Epidemiological data from case-control and cohort studies, and several laboratory investigations indicate that there is a consistent and specific causal association between hepatitis B virus (HBV) and hepatocellular carcinoma and that up to 80% of such cancers are

attributable to this virus. HBV is thus second only to tobacco (cigarette smoking) among the known human carcinogens.

### 1.2.1 *Prospective studies*

Since cohort or prospective studies have markedly strengthened the association between hepatitis B virus infection and hepatocellular carcinoma and have shown high relative risks, these studies were examined in detail. Despite their obvious advantages, there have not been many such studies to determine long-term morbidity and mortality in relation to the various HBV markers. The high cost and the difficulties of long-term follow-up preclude such studies in many places. None the less, the several studies that have been carried out have all shown a marked increased risk for hepatocellular carcinoma among carriers of hepatitis B surface antigen (HBsAg), compared to persons with other HBV markers or with no evidence of previous hepatitis B infection.

These studies have been of three types:

(1) Follow-up of patients with chronic liver disease associated with HBV markers. Such studies have shown that HBsAg-positive patients with chronic hepatitis, cirrhosis, or both, have a much greater risk of developing hepatocellular carcinoma.

(2) Retrospective investigation of HBV serum markers in patients participating in cohort studies. The patients were males of Japanese origin in Hawaii, blood donors in New York, and Eskimos in Alaska. A substantially higher rate of HBsAg was found in the patients who subsequently developed hepatocellular carcinoma than in the controls.

(3) Some prospective studies, in which the patients were unselected for disease but divided into different groups according to their HBV serum markers. This type of study is more useful, but practical considerations make it difficult in most populations. However, two are already published and several others are currently in progress. In one study, with approximately 75 000 man-years of follow-up of about 22 000 middle-aged Chinese males in China (Province of Taiwan) among whom 15% were HBsAg carriers, a 223-fold excess risk for hepatocellular carcinoma among these carriers (compared with Chinese males with no evidence of being carriers) has been demonstrated (1). This study clearly showed that a high risk for this carcinoma was associated with the HBsAg carrier state and not with other HBV markers. A study among railway workers in Japan also

showed a higher risk among HBsAg carriers (2). The annual incidence of hepatocellular carcinoma among HBsAg carriers was similar to that found in the study in China (Province of Taiwan), mentioned above. Other prospective studies in China, Senegal, Singapore, and the United States of America are being conducted, but data are not yet available.

#### 1.2.2 *Mother-to-infant transmission and establishment of the HBsAg carrier state*

Mothers carrying HBV are sometimes highly infectious and in many parts of the world they transmit the infection to their newborn babies. Infection of infants is especially important because a proportion of these infants will become carriers. The number of carriers is inversely related to the age at infection, ranging from approximately 90% in newborns to 10% or less in adults. Infectivity is directly related to the presence of HBsAg in the mother's serum. When mothers are hepatitis B e antigen (HBeAg)-positive, approximately 95% of their newborn children are infected, usually in the perinatal period. The prevalences of HBeAg among HBsAg carriers, and thus the infectivity of mothers for their infants, vary markedly in different circumstances and geographical areas. In Asia, 30–50% of HBsAg-carrier women of childbearing age are HBeAg-positive, and perinatal infections may account for about half of the carriers in the population.

Children of non-carrier mothers can be infected by contact with other children who had been infected by their carrier mothers. Thus, perinatal transmission appears to be the driving force in the maintenance of the high HBsAg carrier rates among people from eastern Asia, for example. The same rates prevail among the Chinese living outside China. In contrast, HBsAg positivity and perinatal transmission are uncommon in Caucasian populations and perinatal transmission is of intermediate frequency in mothers of west-Asian origin or of Afro-Caribbean origin. In Africa, mother-to-infant transmission is also important, but because HBeAg in carrier mothers is less frequent than in Asia, the infection of infants occurs most commonly during early childhood. The timing and mechanism of transmission are of importance in developing strategies for immunization.

It is important to note that mother-to-infant transmission during pregnancy, as opposed to the perinatal period, is rare. The few intrauterine infections that occur are probably due to occasional

leakage of maternal blood into the fetal circulation, because it is known that the virus does not cross the intact placental barrier.

### 1.2.3 *Other etiological factors*

Several factors that may cause hepatocellular carcinoma, independently or in association with HBV infection, have been reported. These include other viruses that can cause hepatitis in man, but there is no evidence that they are involved in human liver cancer.

There is a great deal of experimental evidence that aflatoxins, which are naturally occurring chemicals of fungal origin, are powerful liver carcinogens. It has been shown that people in some areas of the world are frequently exposed to food contaminated with aflatoxin and that a correlation exists between the level of such contamination and the appearance of hepatocellular carcinomas in the areas of the world where the incidence of these cancers is high. Evaluation of the carcinogenic risk of the aflatoxins for man has been reported (3), and the laboratory and field evidence of the health hazards of the aflatoxins has been examined in detail and guidelines for health protection have been recommended (4). Recent reports on the development of radioimmunoassays for the aflatoxins and related metabolites may enable these mycotoxins to be identified in body fluids (5). The direct measurements in individuals, which these methods may make possible, would be a more valuable indicator of exposure than an assessment of food contamination. No other mycotoxins have been incriminated in human liver cancer.

Other substances that have been considered as liver carcinogens include nitrosamines, cycasin, safrole, tannic acid, tannins, and several pyrrolizidine alkaloids. The synthetic chlorinated hydrocarbons including the organochlorine pesticides (such as DDT) and the polychlorinated biphenyls have also been considered; they can cause liver cancer in experimental animals, but there is no evidence of their hepatocarcinogenicity in man.

Androgenic-anabolic steroids are associated with liver angiosarcomas, and steroid contraceptives with benign liver adenomas; there are a few reports that these are occasionally involved in the development of hepatocellular carcinoma.

The pathology of hepatocellular carcinoma, including the role of alcohol, trace elements, and genetic factors and the part played by cirrhosis in the etiology of this cancer, has recently been reviewed (6).

## **2. EARLY DETECTION OF HEPATOCELLULAR CARCINOMA**

Hepatocellular carcinoma is a rapidly fatal disease but the biological behaviour of these tumours in Africa, in the Indian subcontinent, and in Asia appears different. In Africa, these tumours are rapidly growing and occur in early adult life. However, in Asia, some of these tumours grow more slowly. Rising and consistently high levels of alpha-fetoprotein (AFP) in the serum indicate underlying liver cancer in asymptomatic cases. Preliminary results from China and Japan, following resection of these asymptomatic tumours, are promising. However, before such screening is adopted widely in asymptomatic populations, the value of such tests should be assessed by randomized trials; such trials should be carried out only in populations where the appropriate surgical treatment is readily available.

In areas of high incidence of hepatocellular carcinoma, it may be of value to screen regularly, by measurement of serum AFP, persistent hepatitis B carriers in the older age groups. Family members of liver cancer patients, in whom the HBsAg has been detected, could also be screened by AFP estimations.

## **3. MOLECULAR BIOLOGY OF HEPATITIS B VIRUS**

A summary of research on the structure, genetic organization, and biological properties of hepatitis B virus has been published recently (7). One of the most striking recent advances has been the finding of integrated HBV DNA in patients with chronic hepatitis and hepatocellular carcinoma. This integration makes the elimination of HBV DNA in chronic carriers impossible, but prevention may be attained by protection against primary infection. Integration of HBV DNA into human hepatocytes was first detected in a continuous cell line expressing HBsAg. The cell line was derived from a male HBV carrier with hepatocellular carcinoma.

This cell line contains four genome equivalents of HBV DNA per cell, distributed into 4-6 discrete or unique integration sites in host chromosomal DNA, and there is no extra-chromosomal or free viral DNA. The cell line does not contain or produce free virions. It appears that extensive deletions, inversions, duplications and rearrangements have occurred in the HBV genome either during or

after integration into the tumour or during propagation of the cell line. The various integrated forms of HBV DNA in the cells have been cloned, and characterization of the integrated viral sequence and host junction fragments is in progress. Other human liver cancer cell lines containing HBV genome and expressing HBsAg have been described. Using radioactively labelled, cloned HBV DNA probes of high specific activity, HBV DNA has been found consistently in hepatocellular carcinoma from HBsAg carriers. Integration of HBV DNA into one or a number of discrete sites has been reported in almost every tumour in which viral DNA sequences are present. Each tumour shows a unique restriction enzyme pattern of integrated HBV DNA molecules, although bands of similar size have been found in many tumours. In some cases, both free and integrated viral DNA have been found not only in tumour tissue, but also in liver tissue adjacent to the tumour. In such cases, the integration pattern in nonmalignant liver cells may be the same, similar, or completely different from the integration pattern in the tumour. The unique pattern of a limited number of discrete integration sites suggests that all or most of the cells in a given liver tumour specimen are derived from a single progenitor cell which has been stimulated to divide. Integrated HBV DNA has also been found recently in tumours from patients with alcoholic cirrhosis and in the liver of some individuals with no serum markers of HBV indicating present or past HBV infection. This important observation requires further study.

· In HBV carriers with or without histological evidence of liver disease, integration of HBV DNA may be diffuse, in many sites, or present in unique sites of the host genome. Most of these patients have HBsAg and anti-HBe, and it has been proposed that continued HBsAg expression in these patients may be derived from integrated HBV DNA. However, some patients may have HBV DNA in their liver with no expression of HBsAg (latent viral infection), in contrast to those who express HBsAg or virus (persistent viral infection).

· All published studies are consistent with the interpretation that integration of HBV DNA into the hepatocyte genome precedes the development of hepatocellular carcinoma by months or years. The exact time at which integration occurs, the relationship between integration and HBV serum markers, and the frequency with which integration leads to development of hepatocellular carcinoma require further study. From the epidemiological evidence, it would appear that the HBV carrier state precedes by many years the

development of liver cancer. Although these studies do not prove that HBV is oncogenic, the finding of integrated HBV DNA in many hepatocellular carcinomas, and in all such cancers of patients with HBV markers, is highly suggestive.

This is true in spite of the fact that integrated HBV DNA may be found also in non-malignant liver cells during persistent infection.

#### 4. HEPATITIS B-LIKE VIRUSES IN ANIMALS

Detailed consideration of hepatitis B-like viruses in animals will be of outstanding value in the study of the molecular biology of hepatitis B. These viruses share similar morphological and biological properties and genomic organization with the human hepatitis B virus. They have been identified in eastern woodchucks (*Marmota monax*), ground-squirrels (*Spermophilus beecheyi*), and certain ducks (*Anas domesticus*) (8). Some cross-reactivity exists between the viral proteins of different species, and the genomes show the same unique structure as in HBV. A viral DNA polymerase has also been identified in all three animal species. The surface and core antigen genes in the ground-squirrel virus are in the same location and orientation as in the human virus, but there is little sequence homology over long stretches of DNA between woodchuck, ground-squirrel, and duck viruses and human HBV DNA. These viruses do not infect other species, including chimpanzees or man.

Although histological evidence of liver disease caused by these viruses has not been found in ground-squirrels and ducks, acute and chronic inflammatory changes as well as hepatocellular carcinoma have been found frequently in woodchucks. In all but one case of such cancer in carrier woodchucks, the viral DNA was also integrated into the host cellular DNA at a number of discrete sites.

In chimpanzees infected with HBV, both chronic persistent and chronic active hepatitis have been described. Why chimpanzee carriers of HBV do not develop hepatocellular carcinoma is not clear. Whether this simply reflects too short a period of time for these carriers to develop this cancer, a lack of unique HBV DNA integration, or other factors, is also not clear.

Recently a full-length hepatitis virus RNA transcript was identified in the replication cycle of the duck hepatitis virus (DHBV). It has been proposed that this transcript serves as an intermediate for replication of DHBV via a reverse transcriptase-like reaction.

Therefore, hepatitis B-like viruses may have some properties similar to the retroviruses (RNA tumour viruses). This is an important new development that explains how integration of HBV DNA may occur. Proviral DNA of RNA tumour viruses integrates into the host chromosomal DNA as part of its normal replication cycle. Whether this reverse transcriptase activity is the same as, or different from, that observed in the human HBV remains unclear.

## **5. HEPATITIS B VACCINES**

Efforts to grow the virus in tissue culture have been unsuccessful and so far the only vaccines in use are derived from human plasma from carriers of the hepatitis B surface marker.

### **5.1 Standardization and control of hepatitis B vaccines**

The requirements for the production and control of hepatitis B vaccines derived from human plasma, which are of paramount importance for international progress towards the control of hepatitis B virus infection and related diseases, were formulated by a WHO Expert Committee in 1980 (9). Since that time, considerable additional experience has been gained in the production of this vaccine and a large number of doses have been administered. Consequently, the Meeting agreed that there was now an urgent need for a revision of the present WHO Requirements for Hepatitis B Vaccines (9) derived from human plasma.

Work is in progress in several countries on other types of hepatitis B vaccine (see section 5.4). These developments will call for the formulation of separate requirements for their manufacture and control.

The Meeting considered that the use of products derived from human plasma and biologically complex material should be regarded as potentially dangerous and that all possible measures should be taken to ensure the safety of vaccines using such source materials.

A major problem in respect of vaccine control is that the chimpanzee remains the only available animal model for the detection of infectious hepatitis B virus. This primate is in limited supply and its routine use for vaccine control is likely to become increasingly

restricted. As indicated elsewhere in this report, the Meeting considered that the establishment of the consistency of production of the vaccine obtained by a given manufacturing process might be based on testing of the early consecutive batches of vaccine in susceptible human volunteers. However, whenever a new virus inactivation process is employed, it is important that, where possible, the efficacy of the process to inactivate hepatitis B virus preparations should be examined in limited studies using chimpanzees. Chimpanzees may also be required in small numbers to establish the protective efficacy of the new hepatitis B vaccines.

The Meeting stressed the important need for the establishment of international reference preparations of hepatitis B vaccine for use in the control of vaccine potency and purity. The establishment of two reference preparations was recommended:

(1) An aqueous preparation containing pure HBsAg, which is stable in the frozen or lyophilized state and which would provide a reference material for *in vitro* assay systems used for the quantification of HBsAg in the vaccines. This preparation should be established by an international collaborative study and the content of HBsAg should be expressed in micrograms or international units. This preparation would also be of value as a standard for purity as measured by chemical methods.

(2) An adsorbed final vaccine, after tests for potency in man, should be established as a standard for antigenic potency *in vivo*. By international collaborative studies in suitable strains of mice, which must have an appropriate H2 haplotype, this material should be established as a reference preparation for assays of antigenic content.

## **5.2 Inactivation processes for hepatitis B vaccines**

The Meeting discussed the inactivation processes appropriate for the preparation of safe and effective vaccines derived from human plasma. Within the context of the limited use of the licensed vaccines now available, these appear to be safe. All current hepatitis B vaccines and all vaccines to be made in the immediate future, however, will continue to be made using plasma from human hepatitis B carriers as a source of the surface antigen.

It must be appreciated that other infectious agents may also be present in human blood. A cogent current example is the hypothetical agent which is thought to cause the acquired immunodeficiency

syndrome (AIDS) and is characterized by an inversion of the ratio of helper to suppressor T cells (10). This syndrome is associated with the rapid development of Kaposi's sarcoma and opportunistic infections and with a high fatality rate (40%). The hypothetical agent appears to be present in the blood and body fluids and to be transmitted by blood, blood products such as platelets and factor VIII, and close interpersonal transfer of blood and body secretions. The incidence in the USA of the acquired immunodeficiency syndrome is now about 400 cases per year and it has been doubling every six months. The disease is also spreading to other parts of the world.

It was agreed that some of the potential contaminants could be removed by physical methods and the aim should be to start with as pure an antigen as possible. However, since some infectious agents may not be removed by physical methods, biological and/or chemical means must be used to kill all infectious agents.

It was emphasized that since prevention of HBV infection may involve the vaccination of large numbers of persons, including newborn healthy babies, no risks can be permitted with regard to the safety of these vaccines. All participants at the Meeting agreed that should there be an unfortunate accident with the use of hepatitis B vaccine leading to infection, this would delay, perhaps for many years, the ultimate objectives of control of hepatitis B by active immunization and the implementation of any project on the prevention of chronic liver damage and hepatocellular carcinoma.

### **5.3 Safety testing of vaccines**

The Meeting discussed the implications of, and the need for, safety testing of hepatitis B vaccine in chimpanzees, as detailed in the WHO Expert Committee report mentioned above (9).

For the present, as stated in the WHO Requirements for Hepatitis B Vaccine (9), each of 5 batches of vaccine prepared consecutively should be tested in 4 chimpanzees. In the future the WHO Expert Committee on Biological Standardization might wish to consider that before testing in chimpanzees is discouraged, at least 5 batches of vaccine should ideally have been tested both in chimpanzees and in man. Thereafter, it is suggested that each of at least 10 additional batches, also prepared consecutively, should be tested in man before general release of the individual batch. The total of 15 batches would then comprise a consistency series to prove the safety of the vaccine

production process. In all future production, an identical process, like that used in the production of the consistency batches, should be strictly adhered to. Any failure of a batch to pass the test for safety, or any deviation from the standard production process, would invoke the need to introduce adequate measures for safety.

The Expert Committee might also wish to consider that for each of the 15 consecutively produced consistency batches, 20 seronegative volunteers at low risk for hepatitis B (300 volunteers in all) should be given a single dose of the vaccine and observed for a period of 6 months. During this period there would be serological measurements, biochemical tests, and clinical observations to determine whether the vaccine had caused an infection. If, during the 6 months of observation, the 20 volunteers for each of the consistency batches showed no abnormalities, then the process of inactivation that had been used in the production of that batch could be deemed to be safe.

Careful surveillance of the vaccine in general use must be carried out to detect the possible presence of extraneous agents in the vaccine with longer incubation periods, such as the agent causing the acquired immunodeficiency syndrome. The Meeting suggested that WHO's Member States should be informed about the emerging serious public health problem of this syndrome.

In formulating national requirements based on the WHO Requirements for Hepatitis B Vaccine, the national control authority would determine how many batches of vaccine should be tested in man to establish proof of inactivation, and would also determine the number of vaccine batches that should be given to volunteers, who must remain under close observation, before the vaccine could be regarded as safe and released for general use.

The national health authority bears the responsibility for the health of its people and must therefore be responsible for granting permission for whatever clinical trials may be required in man to establish the suitability of each vaccine batch. Such trials should be conducted according to the provisions of the Declaration of Helsinki.

#### **5.4 Vaccines for the future**

In view of the above discussion on safety testing and of the fact that hepatitis B vaccines will continue to be prepared from the

plasma of human carriers for at least the next few years, consideration must be given to the preparation of vaccines from alternative sources with the object of improving safety and reducing cost. It was felt that the search for, and the development of, these alternative sources of safe and potent vaccines should be encouraged. Development work is in progress in several countries on new hepatitis vaccines including hepatitis B polypeptide micelles. Another approach is to use a virus as a vector for DNA coding for the immunogen. Several research groups have replaced the early or late genes of SV40 with DNA coding for another protein. This has been done for HBsAg and reasonable yields obtained. Similarly the hepatocarcinoma cell line, derived from hepatitis B carrier (described in section 3), produces HBsAg. However, because of the association with malignant tissue, this approach may not be acceptable. Cloning of the hepatitis B surface antigen gene in prokaryotes or in eukaryotes has resulted in the expression of surface antigen. This method is promising, provided that the necessary yields and purity of antigen can be obtained at a reasonable cost. Hydrophilic oligopeptides, representing an antigenic determinant of hepatitis B surface antigen, can be synthesized chemically in the laboratory but are of low potency, and a suitable carrier and/or adjuvant will need to be found if such synthetic material is to provide a practical basis for the production of vaccine; these are still only theoretical possibilities and are unlikely to be available before another 5-6 years. The possibility of using an existing viral vaccine, e.g., a modified vaccine strain of vaccinia virus, or a commensal gut organism as a vector for DNA coding for hepatitis was also discussed at the Meeting.

## **6. CURRENT STATUS OF IMMUNIZATION STUDIES FOR PREVENTION OF PERINATAL TRANSMISSION**

Studies of hepatitis B immunoglobulin (HBIG) administered to newborns have been conducted, and without exception were successful in reducing the frequency of HBsAg carriage resulting from perinatal infection. The administration of HBIG immediately after birth to ensure maximum effectiveness is of considerable importance. Several additional doses given during the first year of life have also been shown to increase effectiveness. A reduction of the carrier state of between 40% and 60% was achieved in several large studies. Although many infants receiving HBIG developed immunity, a

Table 1. Studies of the immune response to HBV vaccine in newborn children<sup>a</sup>

Place <sup>b</sup>	HBV vaccine source	No. of infants	Anti-HBs + by 6 months	
			No.	%
Burundi	Pasteur	66	62	94
China (Province of Taiwan)	Merck	31	29	94
Senegal	Pasteur	115	108	94
South Africa	Merck	73	67	92

<sup>a</sup> Excludes infants of HBeAg carrier mothers but includes infants whose mothers are anti-e carriers, and infants who had passively acquired anti-HBs either from the mother or from administered HBIG.

<sup>b</sup> Other studies in newborn children are in progress in several countries including China and the United States of America, but results were not available at the time of the preparation of this report.

significant proportion were nevertheless vulnerable to infection, once the passively acquired antibodies were no longer present. Many of these infants subsequently became infected as a result of continued exposure to their carrier mothers, especially the mothers with HBeAg.

Immunogenicity studies (Table 1) have demonstrated that more than 90% of newborns develop antibodies by 6 months of age in response to two doses of vaccine. These findings are unusual since the response of newborn infants to other vaccines is generally poorer than that of older infants and children. It should be noted that passively acquired antibodies do not interfere with the response to immunization.

Studies in at least nine countries are now in progress to evaluate the immunogenicity and efficacy of HBV vaccine alone or in combination with HBIG. Data from most of these studies have not yet been published, but the results from many of them are expected during the next two years. They involve basically two approaches: (a) use of HBV vaccine only, and (b) use of HBIG plus HBV vaccine. Most investigators expect optimal protection from the latter and numerous dose-time combinations are being evaluated (Table 2).

In a study in Japan of 231 infants, a protection against the carrier state of 90–99% was demonstrated after follow-up examinations for at least 12 months. The infants were given an intravenous or intramuscular injection of HBIG at birth, followed by repeated intramuscular injections of HBIG and active immunization with vaccine on 3 occasions. Studies in China (Province of Taiwan) have shown protection of more than 95% of infants who received both HBIG and vaccine, and approximately 75% of those given only the vaccine at the age of one week.

Table 2. Ongoing studies of immunization against perinatal transmission of HBV to infants of e positive mothers

Place	HBV vaccine source	Immunization schedules being compared <sup>a</sup>					Year when results are expected
		Controls	HBIG only	Vaccine only	HBIG plus vaccine	Approx. number of infants <sup>c</sup>	
Burma	Merck	Yes	No	Yes	No	160	1984
China:							
Qidong	Pasteur	Yes	Yes	No	Yes <sup>b</sup>	100	1983
Province of Taiwan	Merck	Yes	H	Yes	Yes <sup>b</sup>	275	1983
Province of Taiwan	Pasteur	Yes	No	Yes	Yes <sup>b</sup>	150	1983
Federal Republic of Germany	Merck	H	No	No	Yes	.25	—
Hong Kong	Dutch Red Cross	Yes	No	Yes	Yes	120	1984
Italy	Merck	?	No	No	Yes	13	1984
Japan	Kitasato	H	H	No	Yes	384	P
Japan	Kitasato	H	H	No	Yes	500	1983
Japan	Green Cross	H	H	No	Yes	350	1983
Thailand	Merck	?	No	Yes	No	20	—
Sweden	Merck	?	No	No	Yes	2	1984
USA	Merck	H	Yes	No	Yes <sup>b</sup>	124	1983

<sup>a</sup> H = historical controls—i.e., data on controls obtained from previous studies; P = published; ? = no information on whether data on controls were obtained from previous studies or whether controls were included in the study; — = no information available.

<sup>b</sup> More than one schedule being evaluated.

<sup>c</sup> Number enrolled up to January 1983.

## 7. GLOBAL STRATEGIES FOR THE PREVENTION OF HEPATITIS B AND LIVER CANCER BY IMMUNIZATION

The only practical method of achieving widespread effective control of hepatitis B is active immunization. Vaccination strategies must take into consideration geographical patterns in the prevalence of hepatitis B. The prevalence may be conveniently divided into three categories. In low endemic areas, such as North America, Western Europe, and Australia, prevalence of HBsAg in asymptomatic carriers is 0.2–0.5%; while in areas such as Eastern Europe, the Mediterranean region, and South-West Asia, the prevalence varies from 2% to 7%. In high-prevalence areas, such as China, South-East Asia, and tropical Africa, rates of HBsAg carriage may be as high as 15%. Evidence of infection by HBV, as measured by the presence of anti-HBs in serum, shows similar geographical distribution, 4–6%, 20–55%, and 70–95%, respectively, in the three prevalence categories. In countries where hepatitis B is uncommon, infection rarely occurs before adult life. As the overall frequency of infection in the population increases, there is increasing tendency for this infection to occur at an earlier age, and in the areas of highest prevalence the infection occurs mostly in infancy and early childhood. Within each country or geographical region, considerable differences in prevalence may exist between different ethnic and socioeconomic groups.

Strategies for vaccination are shown in Table 3.

In areas of low endemicity, only targeted use of immunization will probably be considered and unless it is deemed essential to identify HBsAg-positive mothers and immunize their infants, the need for immunization products will be small.

The approach for areas of intermediate and high endemicity could be similar, since the decision to embark on immunization by a national authority will be influenced by the logistics of health care delivery and the cost of immunization products as well as the prevalences of hepatitis, the HBsAg carrier state, and hepatocellular carcinoma. The decision to immunize before or after exposure in these areas will depend on the results of the studies outlined in section 6, which will indicate whether or not vaccine alone is effective in preventing the infection and the carrier state of infants. Other factors to take into consideration are the cost and practical problems in carrying out widespread vaccination of all infants compared with

Table 3. Target groups for hepatitis B immunization

Low-endemicity countries		Intermediate- and high-endemicity countries	
Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
(High-risk groups)	Persons with accidental percutaneous exposure	All infants	Infants of HBsAg-positive mothers
Health personnel	Infants of HBsAg-positive mothers		
Dialysis patients	Sexual contacts of acute cases		
Institutionalized patients			
Drug addicts			
Male homosexuals			

Table 4. The use of blood products for the control of hepatitis B

Therapeutic products	Diagnostic reagents	Products for immunization
Blood derivatives	HBsAg anti-HBs anti-HBc HBeAg anti-HBe	Hepatitis B immunoglobulin Hepatitis B vaccine

the demands on the health care systems in identifying HBsAg-positive mothers and subsequently immunizing their children. The former will require large quantities of vaccine, and the latter a countrywide antenatal and laboratory service and a sufficient supply of HBV diagnostic substances and possibly also HBIG. An examination of national vaccination schedules to determine whether HBV vaccine can be given together with other vaccines, such as DTP and/or poliomyelitis vaccine, would also be needed. If HBV vaccine is to be administered as soon as possible after birth, its inclusion in neonatal care must be considered.

Production of hepatitis B vaccine from donor plasma could form a part of a coordinated production of plasma-derived products, all of which are important for the prevention and control of hepatitis B (Table 4). With few exceptions, the purification processes used in the preparation of HBsAg and for the production of diagnostic reagents and vaccine are similar. The transfer of technology to developing countries for the production of vaccines and reagents should be a priority.

## 8. PROPOSALS FOR FUTURE STUDIES

The necessity to evaluate different methods of immunization was stressed. Two groups of subjects were suggested for field trials: neonates and adults who were at risk of developing the hepatitis B carrier state. The design of large-scale immunization campaigns was also considered.

### 8.1 Comparative field studies to assess the effectiveness of immunization

There is a need for field studies using HBV vaccines, involving several hundreds of subjects, before starting on large-scale immunization. These field studies are required in order to determine the optimal use of the vaccine, including timing of injections and the value of HBIG. Since relatively small differences, 85% as opposed to 95%, in the rates of effectiveness of any two vaccine schedules will be of substantial practical importance for large-scale immunization programmes, it is vital that field studies should be designed to detect such differences. Thus, the following are essential:

(a) Participants in comparative field trials should be randomized to ensure that the results are unbiased. If a double-blind randomization is practicable, this should be used.

(b) Sample sizes should be large enough to detect with high probability any small differences. For example, to detect an 85% versus 95% difference in rates of effectiveness with high probability (0.90), 175 subjects are needed in each treatment group using a 2-sided test ( $P=0.05$ ). In studies with smaller numbers of subjects, differences of this magnitude could easily be undetected.

### 8.2 Neonatal immunization

The proposal is to initiate field trials in populations in one or more developing countries where the prevalences of HBV infection, the HBV carrier state, and hepatocellular carcinoma are known to be high. Since epidemiological studies of both this cancer and HBV infection show striking differences between high-risk populations in Africa, South-East Asia and eastern Asia it is necessary to initiate trials in at least two such areas. The trials should be designed to be capable of evaluating the effect of immunization on the risk of this

cancer appearing many years in the future. Other factors influencing the choice of the site for such a trial would be the availability of a medical services infrastructure and background epidemiological data, and a commitment by the government of the host country to provide adequate support to ensure that the main aims of the project could be achieved. It was also considered important that the host country should seek the help of a small group of experts nominated by WHO.

Trials could be initiated with vaccines that have been prepared, tested for safety, and standardized according to the defined WHO Requirements for Hepatitis B Vaccine (9). It is anticipated that within two or three years after commencement of a trial, locally or regionally produced vaccines of required standard should be available.

Immunization of babies would begin as soon as possible after birth. During the trial, serological tests should be carried out to reach the following conclusions:

(a) If anti-HBs only is detected within the first 7 months after immunization, it will be concluded that immunity has been induced and can be expected to last up to 5 years.

(b) If both anti-HBs and anti-HBc are detected at any time after vaccination, it will be concluded that successful immunization has occurred followed by subclinical infection. Immunity would probably be life-long.

(c) If anti-HBc and HBsAg (with or without HBeAg) are detected, this indicates overt HBV infection and hence an immunization failure.

(d) The presence of anti-HBc and the detection of HBsAg for more than 6 months, with or without HBeAg, would indicate not only immunization failure but development of the carrier state.

Successful monitoring of immunization in this way would allow, within 5 years of the initiation of the project, a decision on whether prevention of infection and of the carrier state had occurred, the extent to which subclinical infection had occurred, and the duration of vaccine-induced immunity.

On the basis of the results of the previously described feasibility studies (see section 6), it would be expected that, provided the above favourable conditions were present, immunization could induce a reduction of up to 95% of the naturally acquired infection. At the same time, the incidence of the carrier state would be reduced to the same extent.

To be successful, field trials in developing countries will need considerable external funding. Possible sources for such funds include private foundations, international agencies, cancer agencies, and bilateral agreements between Member States. A donor country in particular will need to know that the recipient country fully supports the field trial proposal and will provide the necessary national support, at least for a period long enough to judge whether the trial has been successful or not.

### **8.3 Adult immunization**

The possibility of conducting immunization trials to prevent liver cancer as a medium-term objective is also proposed.

There is evidence that most HBsAg carriers acquire the condition as the result of early infection. However, there is no evidence that the carrier state arising from an (albeit more rare) late infection, perhaps in adult life, is less likely to progress to hepatocellular carcinoma. Therefore, if a group of male adults could be identified who are at the peak age for this cancer risk but who are negative for HBV markers and are nevertheless at a sufficiently high risk for this infection, they may be suitable for a randomized trial to assess quantitatively the effectiveness of the vaccine in preventing this cancer. In any group preliminarily identified for this purpose, it is important to determine the rate at which hepatitis-negative adults become antigen carriers. This determination must be part of the initial feasibility studies. Only if this rate is sufficiently high would a vaccination trial of reasonable size have adequate statistical power.

### **8.4 Large-scale studies to evaluate the effectiveness of immunization in preventing hepatocellular carcinoma**

Before mass immunization campaigns are carried out, it is desirable to undertake one or more large-scale trials, each of which would probably involve tens of thousands of individuals.

It would also be desirable to conduct similar studies in different areas of the world, because the long-term effectiveness of immunization may vary with factors affecting HBV transmission. These studies will also be useful for assessing the logistic problems associated with the establishment of mass immunization campaigns and their evaluation in different parts of the world.

## **8.5 Design of immunization campaigns**

The Meeting considered that there were good grounds to suppose that vaccination of children with hepatitis B vaccine at birth, or shortly thereafter, would confer long-term protection against the development of hepatocellular carcinoma. Nevertheless, it was recognized that it would be undesirable to consider immunization on a widespread scale without, at the same time, organizing studies that were designed to establish whether or not the vaccine was producing a reduction in the incidence of the carrier state and subsequently cancer. Although the main health benefit expected from large-scale immunization programmes in infants might be a reduction in mortality from chronic liver disease and hepatocellular carcinoma, this is unlikely to occur for some years to come. Earlier endpoints, such as carrier rates after 5 and 10 years, should therefore be incorporated into the study design. The best method of ensuring that a protective effect of immunization could be measured would be to use randomized controlled trials.

In this way, it would be possible to ensure that the groups being compared, both immunized and non-immunized, would be similar with respect to all possible risk factors for this cancer, except for immunization. Such a randomized trial would present ethical problems if sufficient vaccine were available to treat all newborn children. At present, and probably for the next several years, however, this is not the position as the vaccine is expensive and supplies are limited.

## **8.6 Randomization**

In large-scale immunization trials, randomization of individuals would often be impracticable, and it would be better to use randomization at the community, village or hospital level, provided that there are a reasonable number of "communities" (at least 10) in the programme. For example, suppose an infant immunization programme is being planned for a region consisting of 12 communities but that, in the first year of the programme, there is only enough vaccine for 6 of them. Then 6 of the 12 communities can be randomly selected to receive the vaccine in the first year, and the newborn from the other 6 communities in that year can serve as controls.

During the course of the study, the vaccine may become much cheaper and more widely available. In these circumstances, ethical considerations may dictate that all infants should be given the

vaccine. This may not jeopardize the interpretation of the study, since many persons would already have become carriers as infants or young children by the time of general immunization campaigns and would remain at a high risk for hepatocellular carcinoma, and thus serve as controls.

In areas where the vaccine is not allocated on a random basis, it is recommended that careful records should be maintained of all those who are immunized so that an eventual assessment may be made of the effectiveness of the vaccine against this cancer.

### **8.7 Identification and follow-up of subjects**

It is vitally important that, for the satisfactory interpretation of the results, individuals should be correctly identified as having been originally in an immunized or non-immunized group. The logistic problems to ensure this can be considerable, especially in some developing countries. In some societies, children are not named until they have reached a specific age or their names are changed at that time. Date of birth is often uncertain, and it may be necessary to record additional information, such as the names of siblings and their birth order, to be sure of subsequent identification. For the trial to be evaluated adequately it is important to be able to identify members of the non-immunized group, as distinct from non-participants, and it will certainly not be sufficient to identify only the individuals who were given the vaccine.

Even if the participants in the study are identified in a satisfactory manner at birth, it may be necessary to conduct at regular intervals a census of the study population, and to institute some method of continuous monitoring, in order to detect any possible side-effects of the vaccine and other relevant disease events, and possibly also to re-immunize individuals whose apparent immunity has waned.

Migration is common in some places and this may deplete the study population at a rather crucial period. It will probably not be feasible to follow up individuals once they have left the study area, and so the possibility of migration will need to be taken into account in the planning and evaluation of the study.

### **8.8 Associated studies**

The logistics and cost of setting up a long-term hepatitis B vaccine trial may be formidable. If such a trial is started, however, it will

provide excellent opportunities for a wide range of associated investigations aimed at understanding the natural history of HBV infection and associated chronic liver disease. It seems likely that the worldwide distribution of hepatocellular carcinoma cannot be explained solely in terms of the prevalence of HBV infection and that there may be cofactors playing an important part in the etiology of this cancer. Exposure to aflatoxin is a strong candidate for such a role, and this might be studied on a longitudinal basis during a vaccine trial.

## 9. SUMMARY AND CONCLUSIONS

The evidence for an association between the carrier state of hepatitis B virus infection and hepatocellular carcinoma (liver cell cancer) is now sufficiently strong to justify the use of a vaccine against this infection as a means of preventing this cancer. Effective vaccines against hepatitis B are available and have been tested in feasibility studies, and their use in field trials to test their effectiveness against the long-term risk of contracting hepatocellular carcinoma is now possible.

At the present time, only limited quantities of the vaccine derived from human plasma infected by the hepatitis B virus are available, and the development of other types of vaccine was discussed together with the necessary revision of the present requirements for the production of both current and future vaccines against this infection. Since the studies to assess the prevention of this cancer would require the surveillance of subjects for some years, consideration is also given to the design of field studies with short- and medium-term objectives.

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