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VIRAL HEPATITIS IN EUROPE

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Chairman:

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WHO Regional Office for Europe, Copenhagen

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Introduction

The World Health Organization (WHO) has been actively involved in the field of viral hepatitis for about 30 years and has facilitated international collaboration by bringing groups of experts together to share information, often before it has appeared elsewhere, and publishing their deliberations as a series of technical reports and papers. This report summarizes the discussions held by a group of experts convened by the WHO Regional Office for Europe to review the European objectives on hepatitis in the framework of the WHO Global Hepatitis Programme and to consider specific targets which should be included in the Regional strategy.

Several distinct forms of viral hepatitis are recognized, referred to as hepatitis A, hepatitis B, hepatitis D (delta hepatitis), orally-transmitted (epidemic) hepatitis non-A, non-B and parenterally-transmitted hepatitis non-A, non-B. All these various types of viral hepatitis may exist in a community but not be recognized because of the high incidence of asymptomatic or anicteric infections as well as the lack of specific tests for hepatitis non-A, non-B.

The present report does not attempt to review this complex problem in its entirety nor the rapid advances which are being made in research and development. Emphasis, however, is confined to improvement in surveillance of all forms of hepatitis and to strategies for their prevention and control.

Diagnostic criteria for virus hepatitis

General concepts and the methodology of the laboratory diagnosis of viral hepatitis have been reviewed by WHO consultative groups on many occasions and their last consensus was published three years ago (Deinhardt F and Gust I, Bull Wld Hlth Org, 1982, 60, 661-691). As a rule acute viral hepatitis offers little difficulty in diagnosis. History, clinical examination and biochemical tests, including aminotransferases, in most instances lead to a correct diagnosis. Unfortunately, these efforts do not often distinguish the type of hepatitis. Serological testing can distinguish type A, B and D (Delta) hepatitis but there is as yet no reliable serological marker for the different types of hepatitis non A, non B. The latter diagnosis has to be based on exclusion of other factors causing similar types of liver damage.

Diagnosis of hepatitis A infection

Detection of hepatitis A virus (HAV) in faeces:

HAV is excreted in the faeces for 1-2 weeks before the onset of disease Shedding of the virus is relatively brief and may be over by the time the patient seeks medical attention. HAV can be detected in approximately 50% of patients with hepatitis A tested within 1 week of the onset of dark urine. The chance of detecting the virus falls to 10-25% in the second week and less than 10% in the third (Fig. 1). HAV-RNA and HAV antigen may be detected by molecular hybridization or immunological methods respectively. These techniques may be useful for epidemiological studies, detection of the source of infection and environmental pollution, including water supplies, contaminated shellfish and food but are not used in the routine diagnosis.

Demonstration of a rise in serum antibody titre against hepatitis A virus (anti-HAV):

Anti-HAV appears early in the illness and titres rise rapidly. Establishing a diagnosis by demonstrating a four-fold or greater rise in antibody titre is often difficult unless the patient is seen very early.

Demonstration of anti-HAV of the IgM class (anti-HAV IgM):

Anti-HAV IgM is usually detected at the onset of illness and persists for a limited time. This assay is the test of choice for the diagnosis of hepatitis A. Anti-HAV IgG appears more slowly, persists for many years, and indicates

immunity to the disease. A collaborative effort is required to produce reference preparations for anti-HAV and anti-HAV IgM. Standard panels of sera are also required with known titres of various immunoglobulin classes of anti-HAV for use in potency and proficiency testing.

Diagnostic criteria for hepatitis A

Hepatitis A infection is associated with the clinical syndrome of acute hepatitis occurring sporadically or in epidemic form. The diagnosis is confirmed by the demonstration of serum antibodies to the hepatitis A virus of the IgM class (anti-HAV IgM).

Diagnosis of hepatitis B (HB) infection

Exposure to hepatitis B virus (HBV) results usually in an acute self-limiting infection, which may be subclinical or symptomatic. A proportion of infections do not resolve, and these individuals become persistent virus carriers. Persistent carriage of HBV may be asymptomatic or associated with the development of persistent or chronic active hepatitis. Hepatitis B surface antigen (HBsAg) is the primary test for the diagnosis of hepatitis B. Differentiation between acute infection and the carrier state is of clinical and epidemiological importance. A high titre of HBsAg during the acute phase of hepatitis B, with persistence for more than two months, often is followed by the development of chronic hepatitis.

Hepatitis B e antigen (HBeAg) which is derived from the hepatitis B core antigen (HBcAg) can be detected in serum with sensitive radioimmunoassays or enzyme immunoassays in almost all cases of acute hepatitis B infection (Table 1). It normally disappears early during convalescence, usually before HBsAg disappears, and it can also be used to predict the course of the disease. HBeAg in the serum indicates persisting active viral replication, and most sera with a medium to high HBeAg titre also contain measurable HBV DNA polymerase activity and complete virus particles demonstrable by electron microscopy. Blood of such patients must be considered highly infectious. Disappearance of HBeAg and development of antibody to HBeAg (anti-HBe) are in general good prognostic markers but do not guarantee complete clearance of HBV. Anti-HBs appears usually late, sometimes only several months to a year after the disappearance of HBsAg, and indicates immunity.

The marker that is most valuable in the diagnosis of HBV infections is antibody to hepatitis B core antigen (anti-HBc) and in particular anti-HBc of the IgM class (Table 2). With very few exceptions, anti-HBc is present in the early acute phase of the illness. It is detectable for many years and perhaps for life. In epidemiological studies anti-HBc is the most reliable marker for determining HBV prevalence in various populations. Disappearance of anti-HBc associated with persistence of anti-HBs is rare but it does occur, and for epidemiological studies both antibodies should be measured.

In most cases, anti-HBc of the IgM class is the first antibody to increase during, or sometimes shortly before the acute stage of an HBV infection. In general, it persists for several months, up to about one year. Disappearance of anti-HBc IgM may be the most reliable indicator for the clearance of HBV, whereas persistance of anti-HBc IgM seems to be generally associated with persistent or chronic active disease and continuing viral replication. In general, high titres of anti-HBc IgM are found during the acute and convalescent stage of hepatitis B. The titres of anti-HBc IgM observed in persistent or chronic active hepatitis are much lower, although the titres do not correlate with severity of disease.

Hepatitis B DNA polymerase in the serum indicates that viral replication is continuous, but this enzyme is not included in most routine diagnostic tests mainly because it is of limited value for the management of the individual patient. However, under certain circumstances HBV DNA polymerase or measurement of serum HBV DNA are useful for assessment of transmissibility.

A simplified summary of the various serological tests and their significance is given in Tables 1 and 2. However, a diagnosis of the status of an HBV infection cannot be made by laboratory evaluation of HBV markers alone. The patient's history, data obtained by clinical examination, biochemical laboratory tests, and, when indicated, percutaneous needle liver biopsy may also be needed to arrive at an accurate diagnosis in some cases.

Diagnostic criteria for hepatitis B

Hepatitis B infection is identified by the clinical syndrome of acute hepatitis occurring usually following parenteral exposure. The diagnosis is confirmed by the demonstration in serum of the hepatitis B surface antigen (HBsAg) and/or antibodies to the hepatitis B core antigen (anti-HBc) of the IgM class.

Diagnosis of hepatitis Delta infection

Hepatitis Delta (HD) is caused by the hepatitis delta virus (HDV), a defective pathogenic hepatotrophic agent which requires for infection obligatory helper functions provided by hepatitis B virus. The virion consists of a particle of 35-37 nm in diameter that shares the HBsAg coat of HBV. It also contains delta antigen and a very small RNA molecule (approximately 500,000 relative molecular mass). This RNA, which is the genome of HDV, has no molecular homology with HBV-DNA. Disruption of HDV releases soluble delta antigen and RNA. Antibody to HDV (anti-delta or anti-HDV) is produced during the infection. The disease occurs in healthy individuals infected simultaneously by HBV/HDV or in carriers of HBV superinfected with HDV; subjects immune to HBV are protected from infection with HDV.

Hepatitis following simultaneous infection with HBV and HDV is ususally not or marginally more severe than infection with HBV alone, but fulminant hepatitis has also been observed.

Individuals with an acute or chronic HBV infection established prior to exposure to HDV are the most susceptible to infection with HDV. HBV carriers may rescue HDV from inocula of low HBV infectivity, which could not transmit HBV to a susceptible individual and therefore also not HDV. Thus, the risk of infection with HDV is much higher to carriers of HBV than to healthy persons. Superinfection with HDV can be self-limited but more often it develops into chronic disease; only carriers of HBV can acquire chronic hepatitis delta, as persistent HBV infection is a condition for the replication of HDV.

Transmission of HDV is similar to HBV, i.e. parenterally or by close contact. The infection is frequent among intravenous drug abusers and sexually promiscuous individuals. The risk of HDV infection is also high in HBsAg positive haemophiliacs treated with coagulation factors prepared from plasma pools obtained from several thousand donors, but much less in haemophiliacs treated with factors prepared from single blood donations or from plasma pools of only a few selected voluntary donors. HDV is probably maintained in given populations, for example among indigenous populations of South America or in Italian households by the passage of the virus from carriers of HBV and HDV to carriers of HBV alone.

Given the obligatory association of HDV with HBV, control of HBV with a specific HBV vaccine will reduce in due course the population infected persistently with HBV and therefore also reduce HDV infections. The risk of HDV infections represents a further indication for vaccination against HBV of individuals at high risk of this infection.

No specific form of prophylaxis against HDv infection is presently available for carriers of HBsAg. One way of avoiding HDV infection may be limiting the number of intimate contacts with HDV-infected HBsAg-positive individuals because such contacts appear to be an important way of acquiring of HDV infections. Use of blood tested for HBsAg and of blood products derived from single blood donations or from plasma pools of only few donors may help to reduce possible post-transfusion HDV infection. Screening of blood for HBsAg by sensitive techniques obviates the need for testing blood for the presence of hepatitis delta antigen (HDAg).

Laboratory diagnosis: Sensitive radioimmuncassays and enzyme immuncassays have been developed to measure antibodies to HDAg of the IgG and IgM class and specifically of only anti-HD (anti-delta) IgM. Assays for total anti-HD (anti-delta) are commercially available. Intrahepatic HD (delta) antigen can be demonstrated by immunohistochemical methods in frozen as well as in formalin-fixed liver specimens.

Acute HBV/HDV coinfection is diagnosed by the demonstration in serum of the full battery of markers of hepatitis B and in addition by a variable and usually only transient expression of total anti-HD IgG and IgM and/or anti-HD (anti-delta) IgM; a more protracted antibody response is common in drug addicts (Table 3).

HDV superinfection of HBsAg carriers is diagnosed by a brisk anti-HD (anti-delta) response; superinfected long-term carriers presenting with acute HD hepatitis (mimicking hepatitis B) lack anti-HBc IgM. Progression to chronicity is heralded by an increase in titre of anti-HD (anti-delta) IgG and IgM. Both antibodies persist in high titres in chronic HDV infection. HD antigen is seldom detectable in blood and if so only during the early stage of the disease coincident with primary infection; its detection requires treatment of serum with detergent.

Intrahepatic HD antigen is demonstrable in patients with acute and chronic infection; and although HDAg is not detectable in serum of patients with chronic HDV infection, HDV viraemia is demonstrable by molecular hybridization assays using c-DNA probes complementary to the HDV-RNA genome. The finding of low titre total anti-HD (anti-delta) not accompanied by anti-HD (anti-delta) IgM suggests past infection.

Diagnosis of hepatitis non A, non B

Hepatitis type non A, non B (HNANB) is still a diagnosis of exclusion. Type A, B and hepatitis D (delta) can be easily diagnosed by means of serological assays. However, acute hepatitis occurring without these serological markers is not necessarily hepatitis non A, non B. A clinical syndrome indistinguishable from acute viral hepatitis can be caused by other viruses, some bacteria, a variety of drugs, and several non-infective diseases of the liver.

Both Epstein-Barr viruses (EBV) and cytomegalovirus (CMV) can cause acute hepatitis with jaundice. A typical lymphocytosis should be looked for and a slide-test for heterophil antibodies as well as specific tests for EBV markers should be performed to exclude an EBV infection. Cytomegalovirus infection can be diagnosed by testing serum for specific antibodies or by culturing the virus from urine.

Hepatitis non A, non B include the orally transmitted hepatitis A-like (epidemic non A) and the parenterally transmitted hepatitis B-like forms. Attempts were made to propagate an etiological agent of the A-like type in cell cultures but so far without obvious success. Virus particles obtained from such patients were serially passaged in some species of Old World monkeys. Moderate elevation of transaminases and liver pathology compatible with acute viral hepatitis have been observed 12-20 days after inoculation. Virus-like particles capable of reacting with antibody from epidemic hepatitis non A, non B convalescent sera were visualized in the liver cells. Recently, the finding of reverse transcriptase in serum of patients with hepatitis B like cases of hepatitis non A, non B was reported and retrovirus-like particles were demonstrated by electron microscopy in the livers of these patients by. These observations have not yet been confirmed independently.

Diagnostic criteria for hepatitis non A, non B

Hepatitis non A, non B infection is identified by acute hepatitis occurring either sporadically following apparent or inapparent parenteral exposure (the hepatitis B-like types) or occurring in epidemic form following common source outbreaks (the hepatitis A-like type).

There is no serological test at present for hepatitis non A, non B and the diagnosis has to be based on the exclusion of hepatitis A (negative test for anti-HAV IgM) and hepatitis B infection (negative test for HBsAg and anti-HBc IgM). A diagnosis of hepatitis non A, non B also requires exclusion of other causes including hepatotropic viruses like EBV and CMV, hepatotoxic drugs and systemic infections or diseases involving the liver.

Hepatitis non A, non B also may become chronic and the diagnosis in these patients is generally based on the observation of long-standing fluctuating levels of serum aminotransferases and on studies of liver histology.

EPIDEMIOLOGY OF VIRAL HEPATITIS IN EUROPE

Incidence and prevalence data are incomplete to draw a real picture of the epidemiological situation of viral hepatitis in the European region and this stresses the need for improving existing surveillance. Only 8 out of the 32 European countries are reporting hepatitis A, B and non A, non B (NANB) separately and in many countries underreporting is accepted practice. However, from the data given in Table 4 the following conclusions may be drawn.

According to current reported figures more than 120,000 clinical cases of acute clinical viral hepatitis occur annually in European countries (excluding USSR) and the actual total figure certainly surpasses 200,000. The epidemiological pattern of hepatitis A, B, and NANB varies among the European countries as well as among the various population groups within each country. The overall incidence of acute viral hepatitis in Europe increases from Northern and Central to Southern and Eastern countries. Differences also exist as far as the relative frequency of the various types of the disease are concerned. In many countries hepatitis A is the most common type (e.g. USSR and Scandinavian countries), whereas in some others (e.g. Switzerland and F.R.G.) hepatitis B is the most frequent type.

Footnote:

The figures of the total number of cases of hepatitis given in this section are based on the following denominators. Population of the European region of the WHO (excluding Turkey, Morocco, Israel and USSR) considered as 480 Million inhabitants and an average reported incidence rate of acute viral hepatitis as 25 per 100,000 per year. Average distribution of types of hepatitis: Hepatitis A = 40%, Hepatitis B = 40%, Hepatitis non-A, non-B = 15%

Hepatitis A

At present, at least 50,000 cases of clinical hepatitis A occur annually in European countries excluding the USSR. Hepatitis A began to decline in Scandinavian countries, and between 1960 and 1970 also in countries of central Europe, but only recently in Southern and Eastern countries. Present incidence data show that morbidity is stable at a low level in northern and many central European countries. The incidence still declines in areas of high and intermediate levels. While in low incidence countries such as Sweden, Norway, Denmark and Switzerland, hepatitis A often affects adults travelling to high incidence countries outside Europe; the disease occurs mainly in infants and adolescents in south and east European countries.

Hepatitis B

A minimum of 50,000 cases of clinical hepatitis B are estimated to occur annually in European countries excluding the USSR. The prevalence of HBV infections seems to remain constant in most European countries. However, HBV infections have begun to decline in some areas, e.g. in Greece. This might partly be attributed to improvements in conditions of hygiene, screening of blood donors, and other preventive measures against iatrogenic transmission. Sources of infections also differ from country to country. Homosexuality, heterosexual promiscuity and drug addiction account for a significant proportion of HBV infections in Northern and Western European countries. Thus, intravenous drug abusers make up 25% to 40% of the cases in the United Kingdom, FRG, the Scandinavian countries and Switzerland. In contrast, intrafamilial spread, heterosexual and possible iatrogenic transmission account for a great part of infections in southern and some eastern European countries.

Prevalence rates of HBsAg carriers are low in most european countries (0.1-0.6%), but higher in southern and eastern countries (up to 8%). While serological markers of HBV infections are present in 5% to 25% of unselected healthy persons, they range between 30% to 80% in drug addicts, 40% to 70% in male promiscuous homosexuals and up to 50% in health care personnel. HBV infections are also very important since it is estimated that each year 5-30% of the patients with acute disease progress to chronic hepatitis and/or to a chronic HBsAg carrier state. Immunocompromised individuals such as patients on haemodialysis or with renal transplants, hemophiliacs, mongoloids or patients on immunosuppressive therapy are at high risk to develop a chronic HBV infection.

Hepatitis Delta

Surveys of HBsAg carriers with chronic liver damage point to South East Europe as the region with the highest hepatitis D (Delta) prevalence rates, the prevalence is intermediate in the Mediterranean basin and low in Central and Northern Europe where spread is virtually limited to intravenous drug addicts and their contacts.

Hepatitis non A, non B

Hepatitis non A, non B (HNANB) emerges as another important problem in Europe although it constitutes an average only about 15% of all clinically recognized cases. Nevertheless this form of hepatitis is responsible for an estimated 18,000 to 30,000 clinical cases of hepatitis recognized annually. Of these it is estimated that 30% or more develop chronic liver disease. The great majority of post-transfusion hepatitis is attributed at present to HNANB virus. It is also believed that a large proportion of chronic liver disease is caused by the parenterally-transmitted form of hepatitis non A, non B. There is an urgent need for a reliable serological test for the diagnosis of HNANB and for surveillance of the orally-transmitted form of hepatitis non A, non B among travellers and other risk groups.

Changing pattern of spread of hepatitis B and hepatitis A

Traditionally, hepatitis B has been a hazard of blood transfusion, of the use of shared and inadequately sterilized syringes and needles, and of accidental exposure to infected blood. Transmission can occur in the family setting and tends to be related to the degree of crowding in the household and the closeness of the relationship between the individual and the hepatitis B patient or HBV-carrier. It may occur as a result of accidental percutaneous inoculation following the use of shared razors, toothbrushes, bathbrushes, or towels, or by close personal contacts. HBV has been detected in a variety of body secretions and excretions, including saliva, semen, and vaginal fluid, so that infection may be transmitted by intimate contact and by sexual intercourse. In many countries in the European region high rates of transmission of hepatitis B are now limited to male homosexuals with multiple sexual partners and to intravenous drug users.

Care with all techniques in medical and dental settings has decreased significantly the transmission of HBV. Perhaps the best example has been in renal dialysis centres where HBV transmission can be very high. It has been well documented that environmental precautions alone limited transmission in such centres. By the careful use of instruments for individual patients and the testing and segregation of susceptible patients and infectious patients, dialysis centres have been able to stop or at least reduce transmission considerably.

Outside the hospital environment, medical and paramedical practices may transmit HBV infections if the standard of hygiene is poor. Non-professionals who perform minor surgery, such as nose or ear piercing or scarification, seldom take appropriate precautions between treated individuals.

Simple heating of the instruments over a flame would eliminate this unnecessary risk of HBV infection. Similarly, although autoclaving at 120°C for 30 min is preferable, boiling of cleansed needles at 100°C for a minimum of 5 min would reduce the risk of HBV infection. Measures should be taken to limit needle-sharing by intravenous drug users. The use of condoms and other hygienic measures should be propagated for promiscuous individuals.

Improvements in general and personal hygiene have also resulted in significant reduction in the prevalence and spread of hepatitis A, and in many countries in the European region, point-source outbreaks (eg foodborne outbreaks) and infection of travellers to highly endemic areas constitute the majority of cases with clinical hepatitis A.

In general, isolation of patients admitted to hospital with viral hepatitis is not required as long as adequate hygienic conditions are maintained. Incontinent or mentally disturbed patients as well as small children with viral hepatitis should nevertheless be segregated from other patients.

Safety of Normal (Intramuscular and Intravenous) and Specific Immunoglobulins

Normal and Specific Immunoglobulins, prepared for intramuscular administration by cold-ethanol fractionation according to the method of Cohn et al. or equivalent cold ethanol fractination procedures, have a well-established record of safety regarding transmission of viral infections including all forms of hepatitis.

In recent years there has been a growing interest in the manufacture of immunoglobulin preparations intended for intravenous use. A number of reports have associated serum aminotransferase (ALT, AST) elevations with the use of intravenous immunoglobulins. However, this does not necessarily mean that these enzyme elevations are induced by transmission of hepatitis non-A, non-B but at present this possibility cannot be excluded. Preparations of intravenous immunoglobulins may, however, be as safe as the intramuscular immunoglobulins provided that the source material is prepared according to the cold-ethanol methods adhering strictly to the principles of Good Manufacturing Practices.

It is recommended that manufacturers and National Health Authorities should pay particular attention to the safety of newly developed immunoglobulin preparations for intravenous use and the suitability of the manufacturing procedures.

Passive Immunization against Hepatitis A with Normal Immunoglobulin

The intramuscular administration of normal human immunoglobulin, containing at least 100 IU of anti-HAV/ml, before exposure to the virus or early during the incubation period will prevent or attenuate a clinical illness, while not always preventing infection. This may be followed by passive-active immunity, which could confer prolonged immunity on the individual.

The dosage for prophylaxis should be 2 IU anti-HAV/kg body weight; in special cases like pregnant women, and patients with liver diseases doubling of the dosage may be considered.

For travellers to highly endemic areas the following schedule is proposed:

Persons	Period < 3 months	Period > 3 months
< 25 kg	50 IU anti-HAV (0.5 ml)	100 IU anti-HAV (1.0 ml)
25-50 kg	100 IU anti-HAV (1.0 ml)	250 IU anti-HAV (2.5 ml)
> 50 kg	200 IU anti-HAV (2.0 ml)	500 IU anti-HAV (5.0 ml)

After a period of 6 months the administration has to be repeated, unless it is shown that the recipient already has developed his own hepatitis A antibodies.

The value of immunoglobulin in controlling outbreaks of infection in places such as nursery schools has been demonstrated repeatedly. However, the view has been expressed that the use of immunoglobulin on a very large scale is unwise for 3 reasons: (i) individuals with unrecognized anicteric or sub-clinical disease may disseminate the virus in the general community, (ii) the practice appears to be wasteful, and (iii) frequently repeated injections of immunoglobulin in some healthy individuals may be undesirable.

The available methods for titrating anti-HAV make it possible to quantify specific antibody in pooled immunoglobulin preparations. All batches of immunoglobulin should be titrated therefore for anti-HAV content.

The WHO Programme on the Development of Hepatitis A Vaccine

In 1983 the World Health Organization decided to establish a programme for the development of new and improved vaccines against selected infectious diseases.

The establishment of a programme aimed at the development of hepatitis A vaccines was identified as one of the activities of the Vaccine Development Programme. An international steering committee of experts in virology was appointed in 1984 to define the aims of the programme and to consult with WHO on the development of its activities.

The main elements of the initial phase of the hepatitis A vaccine development programme are as follows:

- The collection of well-characterized strains of hepatitis A viruses of diverse geographical and epidemiological origin.

- Studies towards an improved understanding of the pathogenesis and primary sites of replication of the virus.

- Establishment of a panel of monoclonal antibodies against various hepatitis A virus strains for use in virus characterization and antigen analysis.

- Identification of critical antigenic sites of the virus relevant to protective immunity using a combination of selection of non-neutralized mutants in the presence of monoclonal antibodies and recombinant DNA technology.

- Molecular cloning and sequencing of the complete genomes of carefully-selected hepatitis A strains to determine the genetic basis of antigenicity and virulence.

- Rescue of infectious hepatitis A virus by transfection of cDNA clones to construct experimental attenuated vaccine strains by specific modifications of the virus genome.

- Development of experimental vaccines using antigens prepared by controlled gene expression and synthesis of oligopeptides.

- Study of both humoral and cell-mediated immunity to hepatitis A infections.

- Studies of epidemic hepatitis non A, non B virus.

An ultimate goal of the programme is to provide for the development of an inexpensive safe and efficacious vaccine against hepatitis A suitable for general use in susceptible populations. The need for developing hepatitis A vaccines was endorsed and in discussion it was emphasized that recommendations for target groups for routine vaccination, when a vaccine becomes available, may differ according to epidemiological and other considerations in individual countries of the European region of WHO.

IMMUNIZATION AGAINST HEPATITIS B

The high rate of infection with hepatitis B in certain defined populations in industrialized countries and among the general population in many non-industrialized countries stresses the need for effective hepatitis B vaccines (Tables 1 and 2). Hepatitis B may progress to chronic liver disease including chronic persistent and chronic active hepatitis, cirrhosis and hepatocellular carcinoma. Persistent infection occurs in 5-15% of adults after acute infection and in over 90% if infection is acquired early in life. It is conservatively estimated that there are 200 million carriers of hepatitis B worldwide. Immunization against hepatitis B is therefore required for groups at high risk of infection according to epidemiological patterns, socio-economic factors, cultural and sexual practices, and the environments (Tables and 2). In addition, the high rates of infection and perinatal transmission of hepatitis B in some regions dictate the urgency of protective immunization of susceptible women of childbearing age and of infants and particularly of infants born to carrier mothers as the only practical way of interrupting transmission of the infection.

Immunization must also be considered for persons living in certain tropical and non-tropical areas where the prevalence of hepatitis B infection is high, where 10-20% or more of the population may be carriers, and where hepato-cellular carcinoma is common. Hepatocellular carcinoma is one of the 10 most common tumours in the world, with over 250,000 new cases each year, and there is compelling evidence that hepatitis B virus is the cause in up to 80% of cases.

The indications for the use of hepatitis B vaccines in the European Region are accordingly summarised below:

1. Health care personnel with frequent contact with blood or needles, particularly:

   1.1 Personnel, including teaching and training staff, directly involved over a period of time in patient care in those residential institutions for the mentally handicapped where there is a known high incidence of hepatitis.

   1.2 Personnel directly involved in patient care over a period of time, working in units giving treatment to known carriers of hepatitis B infection.

1.3 Personnel directly involved in patient care working in haemodialysis haemophilia and other centres regularly performing maintenance treatment of patients with blood or blood products.

1.4 Laboratory workers regularly exposed to increased risk from infected material.

1.5 Health care personnel on secondment to work in areas of the world where there is a high prevalence of hepatitis B infection, if they are to be directly involved in patient care.

1.6 Dentists and auxiliary dental personnel with direct patient contact.

2. Patients

2.1 Patients on first entry into those residential institutions for the mentally handicapped where there is a known high incidence of hepatitis B.

2.2 Patients treated by maintenance haemodialysis.

2.3 Patients frequently receiving blood or blood products.

2.4. Patients before major surgery who are likely to require a large number of blood transfusions and/or treatment with blood products.

3. Contacts of patients with hepatitis B

The spouses and other sexual contacts of patients with acute hepatitis B or carriers of hepatitis B virus, and other family members in close contact.

4. Other indications for immunization

4.1 Infants born to mothers who are persistent carriers of hepatitis B surface antigen (HBsAg), or are HBsAg-positive as a result of recent infection, particularly if hepatitis B e antigen (HBeAg) is detectable or HBV-positive mothers without antibody to e antigen (anti-HBe). The optimum timing for immunization in conjunction with the administration of hepatitis B immunoglobulin at a contrallateral site, is immediately after birth or within 12 hours (see 5. Immediate protection).

4.2 Health care workers who are accidentally pricked with needles used for patients with hepatitis B. The vaccine may be used alone or in combination with hepatitis B immunoglobulin, as an alternative to passive immunization with hepatitis B immunoglobulin only. Studies on the efficacy of these different schedules of immunization are nearing completion.

5 Immediate protection

Whenever immediate protection is required, as for example for infants born to HBsAg-positive mothers (see 4.1), or following transfer of an individual into a "high risk" setting or after accidental inoculation, active immunization with the vaccine should be combined with simultaneous administration of hepatitis B immunoglobulin at a different site. It has been shown that passive immunization with up to 3 ml (200 international units of anti-HBs per ml) of hepatitis B immunoglobulin does not interfere with an active immune reponse. A single dose of hepatitis B immunoglobulin (usually 3 ml for adults; 0.5-1 ml for the newborn) is sufficient for healthy individuals. If infection has already occurred at the time of the first immunization, virus multiplication is unlikely to be inhibited completely, but severe illness and, most importantly, the development of the carrier state of HBV may be prevented in many individuals and particularly in infants born to carrier mothers.

6. The immune response to the current hepatitis B vaccines is poorer in the elderly and in immunocompromised patients including patients undergoing treatment by maintenance haemodialysis. It is suggested therefore that patients with chronic renal damage be immunized as soon as it appears likely that they will ultimately require treatment by maintenance haemodialysis or will receive a renal transplant.

Consideration should be given to the use of blood from healthy immunized donors with high titres of anti-HBs for use in routine haemodialysis to provide passive protection of such patients who respond poorly to immunization against hepatitis B.

7. Other groups at risk of hepatitis B:

These groups include the following:

7.1 Individuals who frequently change sexual partners, particularly promiscuous male homosexuals and prostitutes.

7.2 Narcotic and intravenous drug abusers.

7.3 Staff at reception centres for refugees and immigrants from areas of the world such as south east Asia where hepatitis B is very common.

7.4 Consideration should also be given to:

long-term prisoners and staff of custodial institutions, ambulance and rescue services and selected police personnel.

Safety and efficacy

Worldwide studies have provided repeated assurances concerning the safety and efficacy of hepatitis B vaccines. Vaccines produced from pooled plasma of hepatitis B surface antigen-positive individuals and meeting the current WHO requirements* have been shown in clinical studies and in more than a million vaccinated individuals to be free of any infectious human pathogens including the human T lymphotropic viruses which are the cause of some human leukemias and acquired immune deficiency syndrome (AIDS). Similar experiences have been obtained with vaccines currently licensed and used in some European Countries. Local reactions after immunization were minor occurring in less than 20% of immunized individuals and consisted of slight swelling and reddening at the site of inoculation. Slight temperature elevations of up to 38°C were observed in only a few individuals. Despite these convincing results on the safety and efficacy of the plasma-derived hepatitis B vaccines, their use has been surprisingly limited (table 3) and should be encouraged. The currently licensed vaccines should be used without waiting for the development of recombinant or synthetic vaccines (see below), in order to limit the further spread of hepatitis B as soon as possible.

A recombinant vaccine produced in yeast is being evaluated; and has shown a similar lack of side reactions. In addition, it does not induce any antibodies against yeast antigens, as determined by very sensitive serological methods. This and similar recombinant vaccines should become available for general use within the next few years.

Pre-screening and testing for immunity

Immunization of immune individuals is not required, and immunization of carriers of HBV is not harmful but ineffective and should be avoided in order to conserve the supply of vaccine and to avoid unnecessary expense. Screening of individuals for HBV markers before immunization depends therefore on the proportion of persons expected to be found positive, the local cost of screening tests and the cost of the vaccine.

*Footnote:

The WHO Requirements for the manufacture and quality control of hepatitis B vaccine help to maintain a high standard of the products and are reviewed and revised from time to time.

The question which antibody or antibodies against hepatitis B virus should be assayed for immunity is important. The two antibodies which indicate recovery from hepatitis B infection are antibodies against a hepatitis B surface antigen (anti-HBs; the neutralizing or protective antibody) and antibodies against the hepatitis B core antigen of the IgG class (anti-HBc IgG; which indicates past replication of the virus). Both these antibodies may be measured by sensitive radio-immunoassays and enzyme immunoassays. Reagents for these tests are available commercially, but they are expensive.

Screening for anti-HBs has a significant number of false-positive reactions, particularly if low titres are accepted. Therefore, reliance on a low cut-off point, for example sample-to-negative ratio (S/N) at a value of 2.1 for a positive reaction, may result in some susceptible persons not being immunized. A S/N value of 10.0 rather than 2.1 should be used as the cut-off point or comparable thresholds if other tests are used. Anti-HBs titres would be better expressed in milli-international units (m IU) per ml measured against a WHO standard preparation of anti-HBs rather than S/N values, but the actual titre of anti-HBs required for protection is not yet established. However, it is estimated that a titre greater than 10 m IU/ml is required for immunity. It should be noted that the presence of anti-HBc would confirm the specificity of a reaction for anti-HBs.

Screening for anti-HBc has also been suggested because it identifies not only those individuals who have undergone a previous infection but also those in the acute phase of infection or in the early convalescent period before anti-HBs production has begun. Individuals negative for anti-HBc should be vaccinated. Individuals positive for anti-HBc would need to be tested in addition for anti-HBs; if negative for anti-HBs, they would have to be tested for HBsAg to determine whether a carrier state exists. If this system of evaluation is used, only those individuals positive for both anti-HBc and anti-HBs should be considered immune. However, this more detailed form of pretesting depends on the availability of adequate laboratory facilities. In addition, testing for HBsAg may not be acceptable to health care personnel and others because of various social, economic and personal reasons. One solution is to examine sera for HBsAg under a strictly confidential code in order to establish whether immunization is required, and secondly in case at a later date the inadvertent finding of a HBsAg carrier state would be ascribed to the vaccine. The cost of pretesting could be reduced by using only one antibody test, either anti-HBs or anti-HBc. When possible it is preferable to carry out both tests to determine prior exposure to HBV. This

will confirm existing immunity in equivocal situations (low titres of anti-HBs or anti-HBc) and avoid the need for repeating either test or performing specificity tests. The policy regarding the need for prescreening, type of tests and the organization of the programme should be based therefore on local circumstances and the expected prevalence of immunity in the population after thorough cost/benefit analysis.

Serological follow-up for anti-HBs of immunized persons is clearly desirable, whenever practicable, to establish whether immunity has been achieved, to assess persistence of immunity and the need for booster immunization. Follow-up should be carried out for high-risk personnel, after completion of the initial course of immunization (see Figure 1).

Immunization schedule

The dose expressed by the manufacturer as ug per ml of HBsAg protein should be used. It is, however, impossible to compare the dosage of different vaccines until a WHO reference hepatitis B surface antigen particle vaccine standard is established.

The schedule of immunization and dose administered should be in accordance with the instructions of the manufacturer and as approved by the National Control Authority. The vaccine must be given preferably by intramuscular injection in the arm at the site of insertion of the deltoid muscle.

International Reference Preparation for Hepatitis B Vaccine

An international collaborative study to establish a preparation of purified, adsorbed HBsAg derived from human plasma as an International Reference Preparation for hepatitis B vaccine has been completed by the National Institute for Biological Standards and Control, London. A report on the study has been prepared for the WHO Expert Committee on Biological Standards which will be asked to agree to the establishment of the standard. The main use of this material will be the standardization of *in vivo* tests in animals for immunogenic potency of vaccines. A further aqueous, purified HBsAg preparation is being assessed as a standard reference material for tests of vaccine purity and for *in vitro* tests for antigen content.

Polypeptide vaccines

Hepatitis B polypeptide vaccines containing specific hepatitis B antigenic determinants of the major non-glycosylated polypeptide of the surface antigen with a molecular weight of 22-24,000 and its glycosylated form, a polypeptide with a molecular weight in the range of 28-30,000 have been prepared. The individual polypeptides of the surface antigen are immunogenic, and the purified 25,000 (designated as p25) and 30,000 (p30) molecular weight polypeptides are effective antigens. The subunit polypeptide vaccines have been tested for safety, immunogenicity and protective efficacy in susceptible chimpanzees.

Polypeptides in monomeric solution in detergent are not a suitable form of vaccine. Consequently, a method of detergent removal which allows membrane polypeptides to reassociate into water-soluble protein micelles has been developed. Protein micelles are aggregates of polypeptides arranged so that the hydrophobic regions are sequestered in the interior of the particles with the hydrophilic residues on the surface so that the resulting particulate forms are water soluble. Comparison of the immunogenicity of hepatitis B micelles with the 22 nm particle vaccine in a mouse potency assay showed that the micells elicited a more vigorous protective surface antibody response than the intact 22 nm spherical antigen particles at all dose levels tested. Safety tests and protective efficacy studies of the micelle vaccine in non-human primates have been completed and clinical trials are in progress. More recently, polypeptide micelles have also been prepared from hepatitis B surface antigen protein expressed by recombinant DNA technology in yeast and in mammalian cells.

Hepatitis B vaccines by recombinant DNA techniques

Recombinant DNA techniques have been used for expressing hepatitis B surface antigen and core antigen in prokaryotic cells (Escherichia coli and Bacillus subtilis) and in eukaryotic cells, such as mutant mouse LM cells, HeLa cells, COS cells, CHO cells, and yeast cells (Saccharomyces cerevisiae).

These studies have established the nucleotide sequence of hepatitis B DNA and the organization of the viral genome. The genome contains four major polypeptide "reading frames".

The "S" gene codes for a protein of molecular weight 25,000 which resembles closely in predicted sequence the non-glycosylated major hepatitis B surface antigen polypeptide (p25) identified by electrophoretic analysis of 22 nm antigen particles purified from plasma of infected individuals. Another "reading frame", the "C" gene, codes for the 21,000 molecular weight viral core polypeptide. The third "reading frame" overlaps the "S" gene, and the fourth partially overlaps the "C" gene.

A continuous upstream region of the open "reading frame" for the S region is termed the pre-surface or the pre-S region. A 35 kilo-Dalton (kD) protein is partially encoded by the pre-S gene and may mediate the binding or interaction of hepatitis B surface antigen and aggregated albumin. Studies are in progress to determine whether the product of pre-S may play a part in protective immunization. The S region and pre-S have recently been expressed experimentally together in eukaryotic cells.

Similarly, there are preliminary observations which suggest that antibody to the core antigen (anti-HBc), and perhaps more likely antibody to the e antigen (anti-HBe), may provide at least partial protection against infection. Hepatitis B virus core antigen has been expressed in high titre in Escherichia coli, and studies are in progress to establish whether antibodies to the core antigen enhance protection or reduce the severity of infection in experimentally infected chimpanzees.

Recombinant hepatitis B vaccines produced in yeast are being evaluated by clinical trials. The results to date indicate that this vaccine is safe, antigenic and free from side-effects (apart from minor local reactions in a proportion of recipients). The immunogenicity, using a dose of 10 ug/ml as compared with 20 ug/ml of the plasma-derived vaccine, is similar to that of the plasma derived vaccine.

The antigen expressed in yeast has been prepared in a micellar form and found to be considerably more antigenic with enhanced immunogenic potency.

Hybrid vaccinia virus vaccine

Potential live vaccines using recombinant vaccinia viruses have been constructed for hepatitis B, and also for herpes simplex, rabies and others. Foreign viral DNA is introduced into the vaccinia DNA by construction of chimaeric genes. This is accomplished by homologous recombination in cells since the large size of the genome of vaccinia virus (185,000 base pairs)

precludes in vitro gene insertion. A chimaeric gene consisting of vaccinia virus promoter sequences ligated to the coding sequence for the desired foreign protein is flanked by vaccinia virus DNA in a plasmid vector. The hepatitis B surface antigen made by vaccinia virus recombinants was similar or identical in its polypeptide composition, buoyant density, sedimentation rate and antigenicity to material obtained from the plasma of hepatitis B carriers.

The recloned vaccinia virus containing hepatitis B surface antigen coding sequences were used to vaccinate rabbits. Typical vaccinia lesions in the skin and high titres of hepatitis B surface antibody in the circulation were produced. Preliminary studies in chimpanzees also indicated the feasibility of using a recombinant vaccinia virus. The vaccinated chimpanzees developed a mild inapparent infection characterised by rapid development of anti-HBs and anti-HBc compatible with a secondary antibody response when challenged intravenously with $10^{3.5}$ chimpanzee ID_{50} units of live hepatitis B virus of a heterologous subtype. Although the chimpanzee had little or no circulating anti-HBs after vaccination, when the recombinant vaccinia was growing in the skin, they appeared immunologically "primed". As a result the chimpanzee had a brisk and sustained secondary antibody response, presumably due to newly synthesized surface antigen which was produced after challenge with live hepatitis B virus. Antibody to HBsAg of the IgG type and the anti-a (the group determinant of the virus) responses were detected early, which is consistent with the anamnestic nature of the response. In this context, it is interesting to note that priming of neutralizing antibody production has been reported by vaccination of rabbits with synthetic peptides of the viral protein I (VPI) of poliovirus, as described below.

At present, however, there is no accepted laboratory marker of attenuation or of virulence of vaccinia virus for man, not only in the host directly inoculated with the virus but also after several passages in the same species. Alterations in the genome of vaccinia virus which are concomitant with the selection of recombinants may alter the virulence of the virus and it has been noted that interruption of the thymidine kinase gene of the virus by insertion of foreign DNA reduced its virulence for mice. Nevertheless, changes in host range or tissue tropism of vaccinia viruses may occur as a result of their genetic modification and these could be caused by changes in the virus envelope as a result of the incorporation of gene products of the foreign viral genes inserted into the vaccinia virus.

The advantages of vaccinia virus recombinants as a vaccine include low cost, ease of administration by multiple pressure or by the scratch technique, vaccine stability, long shelf-life, and the use of polyvalent antigenes. The known adverse reactions with vaccinia virus vaccines are well documented and their incidence and severity must be carefully weighed against the adverse reactions associated with existing vaccines which a new recombinant vaccine might replace. There are also reports of spread of current strains of vaccinia virus to contacts and this may present problems.

Chemically synthesized hepatitis B vaccines

The development of chemically synthesized polypeptide vaccines offers many advantages in attaining the ultimate goal of producing chemically uniform, safe and cheap viral immunogenes to replace many current vaccines which often contain large quantities of irrelevant microbial antigenic determinants, proteins and other material additional to the essential immunogen required for the induction of a protective antibody. The preparation of antibodies against viral proteins using fragments of chemically synthesized peptides mimicing viral amino acid sequences is now a feasible and attractive alternative approach in immunoprophylaxis.
Successful mimicing of determinants of HBsAg using chemically synthesized peptides has been reported by several groups of investigators, both in linear forms and as cyclical peptides. Peptides have been synthesized which retain biological function and appropriate secondary structure, even though they have a limited sequence homology with the natural peptide or are much smaller. In summary, chemical synthesis of small peptides representing specific regions of hepatitis B surface antigen and the pre-S region is now feasible. Antisera to these peptides cross-react with the native surface antigen particles. Synthetic peptides may therefore be employed in due course as vaccines, although mixtures of more than one of the peptides may be required. Of the many questions which remain to be answered, the critical issues are whether antibodies induced by synthetic immunogens will be protective and whether protective immunity will persist. Some of the carrier proteins which had been linked to the synthetic molecules and some of the adjuvants which have been used in animal experiments cannot be used in man, and it is therefore essential to find acceptable and safe materials for covalent linkage, or, alternatively, to synthesize sequences which do not require linkage.

International Biological Reference Materials available from the World Health Organization, on request

(see Lists of International Biological Standards, International Biological Reference Preparations, and International Biological Reference Reagents, World Health Organization, Geneva, 1982)

1) anti-HBs/ad, ay serum, goat: frozen

2) Serum panels for the detection of hepatitis B surface antigen (HBsAg), antibodies to HBsAg (anti-HBs) and its subtypes; human frozen sera:

   a) HBsAg panel (17 sera)

   b) anti-HBs panel (9 sera)

   c) HBsAg subtype panel (7 subtype sera)

   d) HBeAg/anti-HBe panel (3 sera)

   Held and distributed by Centres for Disease Control, U.S. Public Health Service, Atlanta, GA 30333, USA.

3) Additional antisera to HBsAg subtypes

   a) anti-HBs/ad serum, guinea pig

   b) anti-HBs/ay " " "

   c) anti-HBs/ar " rabbit

   d) anti-HBs/ad " goat

   e) anti-HBs/ay " goat

   Held and distributed by the International Laboratory for Biological Standards, Central Laboratory Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066CX Amsterdam, The Netherlands.

4) anti-HBc serum (in preparation)

5) Hepatitis A Immunoglobulin (freeze-dried fractionated human plasma)

   - 100 IU per ampoule

   Held and distributed by the International Laboratory for Biological Standards, Central Laboratory Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066CX, The Netherlands.

6) Hepatitis B Immunoglobulin (freeze-dried fractionated human plasma)

   - 50 IU per ampoule

   Held and distributed by the International Laboratory for Biological Standards, Central Laboratory Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066CX Amsterdam, The Netherlands.

7) Hepatitis A antigen (in preparation)

8) Hepatitis B surface antigen (in preparation)

Recommendations:

- Government health services should institute in accordance with the recommendations of the "Second Conference on Immunization Policies in Europe (1984)" the reporting of all cases of acute viral hepatitis by age and sex and attempt to ensure differential notification of type A, type B, type D, orally transmitted type non A, non B and the parenterally transmitted forms of hepatitis non A, non B.
- The current status of the prevalence of hepatitis A and of immunity against hepatitis A in various age groups in the European region should be evaluated.
- Research on the identification of the agents of both the orally and parenterally transmitted hepatitis non A, non B and the development of specific diagnostic tests should be given a high priority.
- Programmes for the surveillance of orally transmitted hepatitis non A, non B should be encouraged.
- In order to make the best use of scientific expertise and of financial resources and to coordinate all activities aimed at promoting knowledge and control of viral hepatitis in the European region, it is recommended that an adequate number of national centers for viral hepatitis be established and be recognized by the World Health Organization.
- Member states should intensify programmes on the prevention of viral hepatitis by public health measures particularly through improvement of food and personal hygiene as well as health education and specific immunization of high risk groups. There is also a need to encourage the search for and development of specific therapeutic measures.
- Policies for immunization against hepatitis B should be reviewed regularly according to local requirements and circumstances, and revised as further experience and knowledge is acquired.
- Although to date side-effects with the noninfectious plasma-derived hepatitis B vaccines have been minimal and uncommon, and the vaccines licensed for use so far seem to be safe, this is a new type of vaccine and it is important that any adverse reactions should be reported to the National Control Authority and the relevant Medical Committees.
- There are no grounds for limiting the employment of individuals in medical or other professions after they have recovered from hepatitis B regardless of the continued presence of HBsAg, or of persistent carriers whether or

not they are also positive for HBeAg, provided these people have adequate standards of personal hygiene. In some countries, persons who are HBsAg positive are excluded from areas such as dialysis, transplantation, or oncology units where large numbers of immunosuppressed patients are gathered and considerable volumes of blood are used. Under these circumstances, if infection with HBV occurs, the infection could well become endemic in the unit.

In the rare case of a health care worker being shown to have been involved in the transmission of hepatitis B, an investigation of the circumstances involved should be carried out and appropiate measures to prevent further transmission undertaken.

- Additional international reference reagents for standardization and control of tests for the various markers of hepatitis A, hepatitis B and hepatitis Delta should be prepared.
- The WHO Regional office for Europe should improve the information system in the field of viral hepatitis. Short reports and information items may for this purpose be published in the Hepatitis Scientific Memoranda.
- A World Health Organization manual for the currently used routine laboratory tests for determination of markers of viral hepatitis should be developed and be made available to the virological laboratories of all member states in the region.
- Opportunities for training on an individual basis of laboratory personnel should be made available in appropiate laboratories by the member states. Regional or national training courses should be arranged in cases where a specific need might occur.