VIRAL HEPATITIS: RECENT DEVELOPMENTS

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New knowledge about hepatitis viruses and the diseases they cause has accumulated at a remarkable rate over the decade and one-half since the discovery of Australia antigen. The present discussion will emphasize the developments of the past four years since this subject was last reviewed in Medical Grand Rounds. During this time a reliable means for the diagnosis of acute Type A hepatitis has become generally available, the hepatitis A virus has been grown in tissue culture opening the possibility for development of a hepatitis A virus vaccine, the efficacy and safety of hepatitis B vaccines have been demonstrated, understanding of the non-A, non-B hepatitis viruses has increased and a way may have been found to prevent a significant proportion of the post-transfusion hepatitis cases caused by the NANB viruses. These and other issues will be discussed.

THE HEPATITIS VIRUSES

HEPATITIS A VIRUS (HAV)

This agent is believed to be an RNA virus belonging to the Picornavirus family, enterovirus genus. There is no convincing evidence for more than one strain of the virus. HAV is excreted in the stool of acutely infected persons in very high infectious-dose concentrations during the two weeks before the onset of jaundice and is present in lower concentrations for an additional 7-10 days. There is a viremic stage of acute Type A hepatitis, but this is transitory (a matter of days) and a chronic carrier state does not develop. Neither is this virus the cause of chronic liver disease.

Although one tends to think as HAV as the cause of epidemic hepatitis, it is also responsible for 20-40% of sporatic hepatitis cases among urban adults (1).

HEPATITIS B VIRUS (HBV)

HBV is a DNA virus now classified in the new family of Hepadnaviruses, discussed below. Three different particle forms related to the virus tend to occur simultaneously in the circulation of an infected patient (Figure 1). These include the 22 nm spherical and tubular forms and the 42 nm Dane particle which

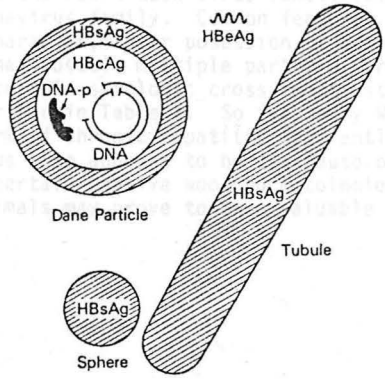

FIG. 1 Structure of the hepatitis B virus associated particles. Abbreviations, see Table 1.

is the actual hepatitis B virion. The relative proportion of Dane particles to the other two forms varies greatly so that, for example, in the case of many healthy HBV carriers despite the strong HBsAg reactivity of their serum due to the presence of the spherical and tubular forms, essentially no Dane particles are present in the circulation. Accordingly, such carriers are unlikely to transmit HBV infection to other persons even by means of inadvertent blood transfusion.

When the HBsAg protein envelope is removed from the Dane particle, core antigen (HBcAg) is exposed. When the Dane particle core is disrupted, e antigen (HBeAg) can be identified as a small (19,000 mw) protein component of core. Also present in the core are the circular, double-stranded DNA, an endogenous DNA polymerase (DNA-P), and a protein kinase enzyme activity.

More than 10 different strains of HBV have been identified. Fortunately, all known strains share a group of common antigens, called "a" antigens, which makes possible their common recognition in the standard HBsAg screening assays. Although it is generally believed that these various strains cause the same spectrum of disease, there are a few indications that this is not always the case; an impressive example was the outbreak of papular acrodermatitis (Gianotti-Crosti Syndrome) which occurred in Japan among children infected with the ayw2 strain of HBV (2). This skin disease is known to be a manifestation of HBV infection in children and has been described most often in southern European countries where ayw2 strain infections are common. Both the disease and the ayw2 strain of HBV have been rare in Japan prior to this outbreak despite the common occurrence of infections due to other HBV strains in that country.

The Hepadnavirus Family

Since its identification the hepatitis B virus has stood alone in the classification of viruses, apparently unrelated to any known virus families. In the past few years, however, three new animal viruses have been identified, and were recognized as sharing many of the peculiar features of HBV (3) (Table 1). Thus, the woodchuck hepatitis virus (WHV) (4), the Beechey ground squirrel hepatitis virus (GSHV) (5), and the Pekin duck virus (DHBV) (6) along with the HBV now constitute the Hepadnavirus family. Common features among these agents are their hepatotropic character, their posession of endogenous DNA-polymerases (unique to the Hepadnaviruses), multiple particle forms present simultaneously in the circulation, certain serologic cross reactivities (7) and several other common features described in Table 1. So far, only WHV has been shown to cause liver disease - a form of chronic hepatitis apparently not progressing to cirrhosis. This virus also appears to be the cause of hepatocellular carcinomas which are common in certain captive woodchuck colonies in the Eastern United States, and these animals may prove to be a valuable experimental model for the

study of this HBV-related oncogenic virus. As in the case of human hepatomas associated with HBV infection (8, 8a), the SNA of woodchuck hepatoma cells has been shown to contain integrated viral DNA (3).

TABLE 1. A COMPARISON OF THE PROPERTIES OF HBV-LIKE VIRUSES

	HBV	WHV	GSHV	DHBV
virions	42 nm spherical 27 nm core p = 1.24 in CsCl DNA polymerase activity	45 nm spherical 27 nm core cross-reactive with HBcAg (10%) p = 1.225 in CsCl DNA polymerase activity	47 nm spherical approx. 30 nm core cross-reactive with HBcAg DNA polymerase activity	40–45 nm spherical 27 nm core (spikes) p = 1.16 in CsCl DNA polymerase activity
genome	DNA circular large single-stranded gap cohesive ends 3,182 base pairs	DNA circular large single-stranded gap cohesive ends 3,308 base pairs some homology with HBV	DNA circular large single-stranded gap cohesive ends 3,250–3,300 base pairs	DNA circular large single-stranded gap cohesive ends approx. 3,000 base pairs
"surface antigen" particles	"HBsAg" numerous in the blood 22 nm spherical and filamentous forms p = 1.19–1.20 in CsCl	"WHsAg" numerous in the blood 20–25 nm spherical and filamentous forms p = 1.18 in CsCl weak cross-reaction with HBsAg (0.1–1%)	"GSHsAg" numerous in the blood 15–25 nm spherical and long filamentous forms p = 1.18 in CsCl weak cross-reaction with HBsAg	"DHBsAg" numerous in the blood 40–60 nm spherical convoluted forms p = 1.14 in CsCl
"natural" host	human	eastern woodchuck (*Marmota monax monax*)	Beechey ground squirrel (*Spermophilus beecheyi*)	Pekin duck and occasionally other breeds (*Anas domesticus*)
distribution in selected populations	0.1–20% persistent infections	16–30% persistent infections	0–50% persistent infections	5–10% persistent infections
transmission	vertical horizontal	?	?	egg transmitted
tissue tropism	liver	liver	liver	liver
associated disease	healthy carriers acute, chronic forms of hepatitis hepatocellular carcinoma	healthy carriers chronic forms of hepatitis hepatocellular carcinoma	healthy carriers ? ?	healthy carriers ? ?

Delta ( ) Antigen

In 1977, Rizzetto et al, using immune fluorescence microscopy, described a new antigen in liver cell nuclei of HBsAg positive patients (9). This reactivity called delta antigen has never been found in tissue of HBsAg-negative persons. Electron microscopic studies have failed to demonstrate components of HBV in delta antigen positive nuclei (10). It has been shown that the delta antigen and antibody system is distinct from known antigen antibody systems of HBV (11). Prevalence studies of delta antigen and antibody in human populations (12) and transmission experiments in chimpanzees (13) indicated that this antigen is associated with a transmissable pathogenic agent that is either a mutant form of HBV or another agent that requires helper functions of HBV for its expression, and which interferes with HBV replication.

Delta antigen extracted from hepatocyte nuclei has been characterized as a protein of 68,000 mw (10). Recently delta antigen has been identified in sera of infected chimpanzees in a discrete subpopulation of HBsAg particles, and in those particles is associated with low molecular weight RNA (14). These delta antigen-containing HBsAg particles are intermediate in size (35-37 nm) and buoyant density between Dane particles and the 22 nm HBsAg spherules. The RNA, which was co-purified with the delta antigen-containing HBsAg particles, is smaller than the genome of known RNA viruses but larger than the viroids of higher plants.

The HBsAg protein of the 36 nm delta-containing particles from the chimpanzee serum studied by Rizzetto (14) appeared to be subtype ad, the same type as the HBV chronically infecting this animal, and different from the ay type of virus carried by the human donor of the delta antigen-containing serum used to infect the chimpanzee. Therefore, it appeared that the HBsAg surface of the delta antigen-associated particle was contributed by the HBV of the host animal and not that of the donor.

These delta antigen-associated HBsAg particles now have also been described in the serum of a human HBsAg carrier (15).

Epidemiologic studies have demonstrated delta antigen and antibody most frequently in Italy, among multitransfused HBsAg carriers and drug addicts (12).

It has been determined that delta antigen is preserved in formalin-fixed paraffin sections (16). In a study of liver biopsies from 571 Swiss patients, 365 of whom were HBsAg positive, delta antigen was identified by immunoflorescence microscopy in 10 biopsies. All 10 were from HBsAg positive patients, 9 with chronic active hepatitis and one with chronic persistent hepatitis; 5 of these patients were drug abusers. In follow-up biopsies, delta antigen persisted as long as 6 years.

In another study, delta antigen was found in liver biopsies from 4 of 20 Italian children with CAH-B (17). The delta antigen positive children appeared to have a more aggressive form of disease.

NON-A, NON-B HEPATITIS VIRUSES

It is now evident that there are at least 3 different NANB hepatitis viruses. Two of these are believed to be responsible for most cases of transfusion-associated hepatitis, and are similar in many respects (but probably not closely related to) hepatitis B virus. A third NANB agent more closely resembles hepatitis A virus.

It has been suggested that the two (or more) parenterally transmitted NANB viruses can be distinguished on the basis of the average incubation period of the hepatitis infections they cause, the pattern of transaminase elevations accompanying these hepatitis episodes, the propensity to cause chronic hepatitis and the nature of the electron microscopic changes which they produce in liver tissue. None of these claims of distinguishing features have yet been convincingly substantiated. The best evidence for two different agents, however, comes from cross-challenge experiments in chimpanzees wherein animals infected by inoculation with one serum pool were later shown to be immune to reinfection

with the same serum, but did develop hepatitis when exposed to a second serum pool which evidently contained a different virus. (18).

At least 10 different laboratories have reported finding virus-like particles by EM in liver tissue or serum of infected chimpanzees and humans. The particles have ranged in size from 20 to 80 nm. It remains to be determined whether any of these particles actually represent hepatitis viruses. As Tabor has pointed out "unfortunately, it is very easy to find things that look like viruses in serum or liver tissue" (19).

In addition, a number of laboratories have reported the development of serologic assays for NANB viral antigens. These are discussed in a following section.

THE HEPATITIS A VIRUS-LIKE NANB VIRUS

In recent decades there have been periodic reports of massive outbreaks of acute hepatitis in India. Because of the consistent clinical and laboratory features, epidemic presentation, and strong evidence of a fecal-oral mode of transmission, these have been regarded as Type A ("infectious") hepatitis outbreaks. Until recently, however, certain features of these cases have proved difficult to explain. For one thing, the disease was as common or more so in adults than in children. This is the reverse of the typical age distribution of epidemic hepatitis A cases elsewhere in which adult cases have been less common because of pre-existing immunity to the virus, among other reasons. This aberrant pattern of the Indian epidemics has been attributed to rather massive viral challenge which was thought to have overwhelmed the waning hepatitis A immunity in adults (Mosley J.; Personal Communication, 1975). However, since hepatitis A is highly endemic in India, the average adult's immunity ought to be sustained at a high level by recurrent "booster" exposures to the virus. (It would be of interest to examine anti-HAV titers in children and adults in such populations for direct evidence on this question.)

Another atypical feature of the Indian epidemic hepatitis cases has been the alarmingly high rate of severe, often fatal, illness among pregnant women (20). While there is evidence that HAV can produce fulminant hepatitis on occasion (21), careful studies of pregnant women with viral hepatitis in Western countries indicated no such increased risks (22). It was suggested that malnutrition may have explained the more severe course in Indian women.

In early 1979 Khuroo evaluated a common-source, waterborne hepatitis epidemic in the Kashmir Valley in India which was typical of other, earlier outbreaks in that region (23). Serologic evaluation for HAV and HBV markers revealed the expected high prevalence of established immunity to hepatitis A (i.e., IgG anti-HAV) but, no evidence for acute infection with either HAV (IgM anti-HAV) or HBV. The data provided convincing evidence for a NANB virus, probably distinct from those known to cause post-transfusion hepatitis.

The question of the increased severity of epidemic hepatitis among pregnant women in India was addressed in another study of the Kashmir outbreak (24). Among 18 women who developed hepatitis in the third trimester of pregnancy, 9 had a fulminant course and 6 of them died. In contrast, there were no fulminant cases among 18 women in the first and second trimester, or among 71 non-pregnant women with hepatitis (Table 2).

TABLE 2 **Incidence and Fulminant Rates of Icteric Viral Hepatitis in Pregnant Women, Nonpregnant Women and Men**

	No.	Viral Hepatitis (no.)	
		Total	Fulminant
Men (15–45 yr old)	3,822	107 (2.8)	3 (2.8)*
Nonpregnant women (15–45 yr old)	2,350	71 (2.1)	0
Pregnant women			
1st trimester	34	3 (8.8)	0
2nd trimester	77	15 (19.4)	0
3rd trimester	97	18 (18.6)	9 (44.4)†

NOTE: Figures in parentheses are percents.
* Three fatal cases of hepatitis.
† Six fatal cases of hepatitis.

To what extent the high fatality rate of acute hepatitis among pregnant women in underdeveloped countries is due to viral factors or to malnutrition, or both, remains to be determined.

THE USE OF SEROLOGIC TESTS FOR HEPATITIS VIRUSES

Sensitive radioimmunoassays (RIAs) are now available for the detection of hepatitis B surface antigen (HBsAg), surfaces antibody (anti-HBs), core antibody (anti-HBc), e antigen and antibody (HBeAg and anti-HBe) and hepatitis A antibody (anti-HAV). In addition to these RIAs, an enzyme-linked immunoassay (ELISA) is also available for HBsAg and, in all likelihood, eventually will be for each of the other hepatitis virus markers. The sensitivity of the antigen assays probably will be increased further by the use of selected mixtures of high affinity monoclonal antibodies and in their manufacture.

The sequence in which the various HBV markers develop during the course of infection is shown in Figure 2 (24a).

A number of commercial clinical laboratories offer "hepatitis screening panels" which consists of an irrational and therefore wasteful combination of tests. Typically included are assays for HBsAg, anti-HBs, anti-HBc, and anti-HAV. In most cases the tests would better be ordered individually, in a sequence such that only those likely to provide useful information would be obtained.

Opinions differ somewhat on the optimal use of these tests, and indications for them vary according to the clinical problem being evaluated. The following comments are suggested as a guide for their application.

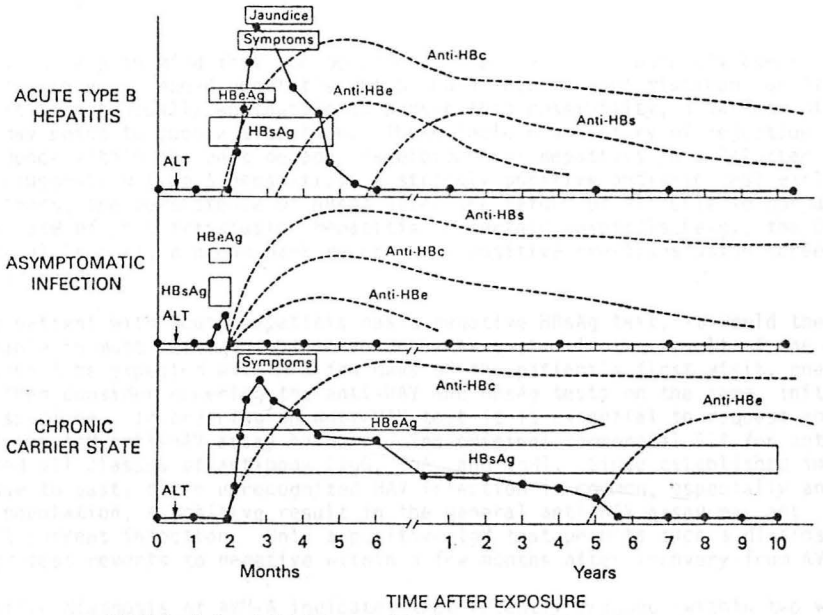

FIG. 2 Serologic and clinical features of the three types of hepatitis B virus infection. Abbreviations, see Table 1. ALT, alanine aminotransferase.

ACUTE VIRAL HEPATITIS (AVH)

For most patients with sporadic (i.e., non-epidemic) acute hepatitis an HBsAg test should be ordered. A positive test essentially defines the etiology of the disease, indicates the need to recommend hepatitis B immune globulin (HBIG) prophylaxis for spouses or other regular, recent sexual contacts and, at the same time, determines that there is no indication for immunoglubulin prophylaxis for other contacts. In addition, the diagnosis of acute Type B hepatitis alerts the physician to the 5-10% probability that the patient will later develop chronic hepatitis and that it will therefore be necessary to demonstrate, in addition to normalization of the "liver function tests" (LFTs) conversion of the HBsAg test to negative before discharging the patient from further follow-up.

e antigen is believed to be present early in the course of all patients with AVH-B, and its persistence in such patients beyond 10 weeks of illness makes it probable that the disease will progress to chronicity (25). On the other hand, the earlier loss of HBeAg, often followed within a week by the appearance of anti-HBe, provides strong evidence that the infection will be self-limited. This seroconversion may occur before other signs of recovery are evident. However, it is not recommended that the HBeAg-anti-Hbe tests be monitored routinely during the course of acute hepatitis, since, lacking specific therapy to influence the progression to chronic hepatitis, knowledge of the patient's e-status is not likely to alter the management of the patient.

One has to keep in mind that, on occasion, unrecognized chronic HBV carriers may present with acute non-B hepatitis which could therefore be mistaken for AVH-B. While it is not usually worthwhile to pursue this possibility, a variety of clues may point to such a situation. These include a history of rejection as a blood donor within the past decade, development of hepatitis in a "cluster" of cases (suggesting Type A hepatitis), a strongly positive anti-HBe test early in the illness, the persistence of HBsAg after the return of all LFTs to normal, or in the case of post-transfusion hepatitis in certain hospitals (e.g., the Dallas VA Medical Center), a blood bank record of a positive pre-transfusion screening test for HBsAg.

If the patient with acute hepatitis has a negative HBsAg test, it would then be reasonable in most cases to obtain an anti-HAV test. If the result of the HBsAg test cannot be expected within a few days of the patient's first visit, one could then consider ordering the anti-HAV and HBsAg tests on the same, initial blood specimen. In ordering an anti-HAV test it is essential to request that (only) the IgM-anti-HAV assay be done. The original commercial RIA for anti-HAV detected all classes of antibody (IgG, IgA, and IgM). Since established immunity due to past, often unrecognized HAV infection is common, especially an the adult population, a positive result in the general anti-HAV assay may not reflect current infection. Only a positive IgM test permits such a diagnosis. The IgM test reverts to negative within a few months after recovery from AVH-A.

A specific diagnosis of AVH-A indicates that recently exposed (within two weeks) household contacts of the patient, and certain other contacts, should recieve a prophylactic injection of human Normal Immune Globulin (ordinary gamma globulin). It also indicates that the patient is not at risk of developing chronic hepatitis or a chronic viral carrier state. The diagnosis of AVH-A would probably make it reasonable for a physician to terminate follow-up care once the patient has become asymptomatic and has returned to full activity, even if the transaminase tests and other LFTs have not yet returned to normal. This would not be appropriate management for the patient with type B or NANB-AVH since those patients, at such a stage of incomplete recovery, are still at some risk of developing chronic hepatitis.

If the patient with AVH has negative tests for both HBsAg and IgM-anti-HAV, the possibility of a NANB virus infection is raised. Since the diagnosis of NANB hepatitis must at present be one of exclusion, it may first be necessary to investigate the possibility of an "occult" hepatitis B virus infection in an HBsAg-negative patient before making the diagnosis of NANB hepatitis. It has been suggested that about 5% of all anti-B patients are HBsAg negative. The diagnosis is indicated by the combination of a positive core antibody test and a negative surface antibody test. (If both of the anti-HBc and anti-HBs tests are positive, this usually means that the patient had been infected with HBV in the past and had recovered and that HBV is probably not the cause of the current illness.) The diagnosis of occult HBV infection is further supported by the development of anti-HBs after clinical recovery.

Such cryptic HBV infection has also been recognized in some patients with chronic hepatitis by virtue of an isolated positive anti-HBc test. Other persons positive for core antibody alone may be healthy chronic carriers of the virus. However, to make the matter even more confusing, it is apparent that among asymptomatic blood donors who are positive for anti-HBc alone (negative for HBsAg and anti-HBs), only a minority are actually infected with HBV, the

remainder apparently representing cases where, following recovery from a remote past infection, the patient either never developed anti-HBs, or if he had, had subsequently lost this antibody (26). Despite the routine screening of blood donors by extremely sensitive HBsAg assays, roughly 10% of transfusion-associated hepatitis cases are still due to HBV infection, and it is possible that the majority of responsible blood donors have occult HBV infections (27). Although at least some of these carriers could be identified if all HBsAg-negative blood donors were further screened for anti-HBc and anti-HBs with exclusion of those donors who are positive for core antibody alone. But this has not been required of blood banks because, as noted above, only a minority of persons having core antibody alone are actively infected. Therefore such screening, which would add substantially to the cost of blood processing, would result in the waste of significant quantities of safe blood. Recently there has been interest in refining assays for IgM core antibody. This antibody may be a marker for active HBV replication, even in the absence of HBsAg. Its identification may enable the specific recognition of active cryptic HBV infections (26). Conceivably, in the future such an assay could be employed along with the HBsAg test to screen blood donors.

CHRONIC HEPATITIS

In the evaluation of a patient with chronic hepatitis, the HBV status should be determined with an HBsAg test and if that is negative, with anti-HBc plus anti-HBs tests to rule out cryptic infection. The recognition of HBV as the cause of chronic active hepatitis may eventually have therapeutic implications which are discussed in a following section.

If the HBV tests are negative, and chronic hepatitis due to other recognized factors can be excluded, a tentative diagnosis of chronic NANB infection can be made. This is particularly true if the illness has appeared at an appropriate interval after blood transfusion.

A positive e antigen test in a patient with chronic Type B hepatitis tends to correlate with more active disease, which is often in the earlier years of its natural course. In addition, it indicates that the patient is, relative to other HBsAg-positive persons, at maximal risk of transmitting infection to other persons. This is likely to be of particular importance for persons such as dentists and surgeons who are carriers of this virus.

When chronic hepatitis B patients become e antigen negative they usually develop anti-e thereafter. This tends to occur at a time when other indicators show that the disease has become less active. At this point it may be progressing to a completely inactive stage, perhaps even leading to conversion of the HBsAg tests to negative.

DETERMINATION OF IMMUNITY TO HEPATITIS INFECTION

Except for unusual cases such as persons working in institutions for the care of mentally retarded children and primate caretakers, there is little reason to test for established immunity to HAV infection. The "general" anti-HAV test which detects all classes of anti-HAV including IgG antibody, and not the IgM-anti-HAV test, would be appropriate for this purpose.

It is often important to know the immune status of apparently healthy persons who are at increased risk of infection with hepatitis B virus. This applies to family members, especially spouses, of chronic HBV carriers, the staff and HBsAg negative patients in hemodialysis units, dentists, surgeons, etc. Demonstration of immunity tends to relieve the anxiety of persons at risk, sometimes permits the "triage" of HBV infected patients to the care of immune medical and dental personnel, and eliminates the exposed person's apprehension about impending infection; the need for expensive HBIG injections in persons who have had overt HBV exposures is obviated.

HBV immunity is essentially proven by the detection of any of the HBV markers. It would be reasonable to test first for anti-HBs, and if it is negative, for anti-HBc. If both of these tests are negative, the individual is probably susceptible to HBV infection. If only the core antibody test is positive, the subject should then be tested for HBsAg, since he may actually be a carrier of the virus.

SEROLOGIC TESTS FOR NANB VIRUSES

Considering that Blumberg succeeded in identifying the hepatitis B virus by use of the simple agar gel diffusion technique (and was awarded the Nobel Prize for this accomplishment) it was not surprising that this method has been applied to the search for NANB viruses. But, it was quite surprising to many interested observers that a number of serious investigators reported positive results in such efforts (28, 29, 30). It had been possible to identify the HBV in serum because of its property, unique among human viruses, of inducing the host liver cells to produce a massive excess of capsular protein in the form of the 22 nm spherules and tubules, as discussed above. It would seem that the only hope of identifying the new hepatitis virus by such an insensitive assay technique would be in finding an HBV-related agent, i.e., another human hepadnavirus.

The failure of efforts to establish radioimmunoassays using the reagents which were said to have produced specific precipitin lines in agar plates has been puzzling.

In an effort to evaluate the specificity of the various NANB assays discussed in the literature, Dr. Harvey Alter of the NIH Clinical Center Blood Bank prepared a coded panel of 36 serum samples (31). These represented duplicate aliquots of 8 specimens proven to contain infectious NANB virus by chimpanzee inoculation studies, 3 additional specimens designated "Probable Infectious" obtained from a single patient with NANB post-transfusion hepatitis, and 7 negative specimens including 2 liver disease controls (sera from one primary biliary cirrhosis patient and one alcoholic liver disease patient) and 5 highly pedigreed donors with a documented history of repeated safe blood donations. The panel was submitted to 7 different laboratories which had previously reported the establishment of serologic assays for NANB viruses. The laboratories included those of W. Arnold, Berlin; F. Deinhardt and G. Frosner, Munich; L. Overby, Chicago; R. Shirachi and N. Ishida, Sendai; E. Tabor and R. Gerety, Bethesda; R. Thomssen, Gottingen; and C. Trepo and L. Vitvitski, Lyon. Sadly, when the code was broken, it became evident that none of the 6 laboratories employing agar gel diffusion, counterelectrophoresis, or immunoflorescence assays had a true test for NANB virus (Table 3).

Table 3

NANBV PANEL II (ALTER;NIH)

Specimens tested by AGD, CEP or IF assays

LAB	PRESUMED POSITIVE (N = 11)	PRESUMED NEGATIVE (N = 7)	STATISTICAL SIGNIFICANCE
1	0	0	NS
2	0	0	NS
3	1	2	NS
4	2	0	NS
5	4	5	NS
6	0	1	NS

By contrast, however, the one laboratory using a solid-phase RIA method (W. Arnold, Berlin) demonstrated rather encouraging results; they detected 5 of the 8 proven infectious sera and 2 of 3 probably infectious sera, with no false-positive results and with 100% agreement for the paired (duplicate) samples. The performance of this RIA was statistically significant as a true test for NANB virus at the p-0.02 level by Fisher's exact test. Of further interest, this assay recognized representatives of both the Hutchinson (H) and Foross (F) inocula, which are believed to contain 2 different strains of NANB virus.

TREATMENT OF VIRAL HEPATITIS

No specific mode of therapy for any type of acute viral hepatitis has been proven beneficial, and no means is known to prevent the progression of acute Type B or NANB hepatitis to chronic hepatitis.

Although certain studies have convincingly demonstrated the benefits of corticosteroid therapy for "chronic active hepatitis" it appears that it was primarily, and perhaps only, the autoimmune-or "lupoid" hepatitis patients within those study populations who had responded to treatment (32).

Whether patients with Type B chronic active hepatitis (CAH-B) ever repond favorably to immunosuppresive therapy is uncertain, but it is clear that in the majority of cases they do not. A recent controlled study in Hong Kong of ethnic Chinese patients with CAH-B suggested that corticosteroid therapy, on balance, had a detrimental effect on the course of these patients (33). Despite the possibility that orientals may respond differently to HBV infections than persons of other races (34), and the recognition that the majority of patients in Hong Kong are infected with HBV strains rarely found in Western countries, this study provided the best evidence to date that, at least for most CAH-B patients, steroid therapy is not indicated.

It has been demonstrated that corticosteroid therapy increases the titers of serum HBsAg and HBcAg (Dane particles) in patients with CAH-B (35). Of greater importance may be the recent reocognition that withdrawal of immunosuppressive therapy from CAH-B patients may actually be of distinct therapeutic benefit.

Muller et al studied 15 patients with CAH-B who had been receiving immunosuppresive therapy for at least 2 years (36). Two had been on prednisone, two on azathioprine, and 11 on a combination of these drugs. Treatment was withdrawn abruptly and each patient was followed monthly for the next seven months. While none of the patients became HBsAg-negative during this time, 5 of the 11 HBeAg-positive patients became HBeAg-negative. In the 3 HBeAg-positive patients with ALT levels above 50 while on therapy (60, 90, and 200 IU), the levels fell to less than 50 when the HBeAg became negative off therapy.

It could be argued that after cessation of treatment one simply observes the subsidence of disease activity which would otherwise have occurred in the absence of therapy by this time in the natural course of the disease. Still, the possibility of inducing the loss of HBeAg with the probable reduction of infectious risk by means of immunosuppressive treatment followed by abrupt withdrawal may deserve further study. Such a therapeutic maneuver may be of special benefit to certain HBsAg/HBeAg-positive dentists, surgeons, and others who are at particularly high risk of transmitting infection to their patients.

Our recent experience has suggested the possibility that withdrawal of immunosuppression may on occasion even lead to resolution of active HBV infection in certain patients. A 38 year old man who had been treated with cyclophosphamide for severe HBV-related polyarteritis nodosa developed agranulocytosis after 6 months of treatment. At that time the polyarteritis was inactive and there was evidence of mild chronic liver disease. Five weeks after stopping treatment, he developed the clinical picture of acute viral hepatitis, with a serum bilirubin level of 15.0 mg/dl, SGOT 1180 IU, and a negative IgM anti-HAV test. Thereafter his condition progressively improved. HBeAg which was consistently positive from the beginning of his polyarteritis, became negative one month after stopping Cytoxan and remained so on each of several later determinations. After resolution of the acute hepatitis-like episode the serum became anti-HBe positive. A few weeks later his serum became HBsAg negative. Four further HBsAg tests over the next two months have all been negative, and recently anti-HBs has been detected in his serum for the first time. In this case it would appear that resurgent immunocompetence after cessation of Cytoxan therapy led to an immunologic assault on the HBV antigens in the liver, with resultant transitory liver injury and eventual elimination of the active HBV infection.

Controlled studies of NANB-CAH treatment have not yet been reported. Anecdotal experiences have not been encouraging, and there is skepticism that any form of viral CAH is improved by steroid therapy (37).

INTERFERON AND ADENINE ARABINOSIDE (Ara-A) THERAPY OF CAH-B

Several agents have been suggested, largely on theoretical grounds, as specific therapy for chronic HBV infections. Among these interferon and, more recently, Ara-A have received the greatest attention in clinical trials. The most extensive studies with these agents have been carried out by Merigan and associates at Stanford University.

In their earlier studies with human leukocyte interferon (HLI) the Stanford investigators noted the disappearance of all markers of active HBV infection in 2 of 7 patients treated with HLI alone. One other patient showed a permanent disappearance of circulating Dane particles, and a sustained reduction of HBsAg titer (38).

Because of the great cost of interferon, as well as its incomplete efficacy, the Stanford investigators (39) and those at the Royal Free Hospital in London (40) evaluated adenine arabinoside (Ara-A) therapy of CAH-B. The initial studies showed that Ara-A was capable of producing a permanent reduction to negative of Dane particle markers (DNA-polymerase, HBeAg, and liver HBcAg) (38). But this was accomplished at the cost of rather alarming toxicity manifested as GI symptoms, weight loss, tremor, myoclonus, ataxia, disorientation, granulocytopenia, and thrombocytopenia) (41).

It was hoped that the problems presented by either interferon or Ara-A used alone could be reduced by using the two agents in combination in modified doses (42). When administered simultaneously side effects, especially neurotoxicity, remained a major problem. However, the preferred regimen of the Stanford group at present, administration of these drugs in alternating sequence, may produce the most favorable therapeutic to toxic ratio.

The use of Ara-A has been made easier by the availability of a water soluble monophosphate derivative, Ara-AMP, which can be given by intramuscular injection rather than by prolonged intravenous infusion as required for Ara-A.

The Stanford group is currently preparing to carry out a controlled study of interferon/Ara-AMP therapy of CAH-B. It is evident such controlled studies will be necessary before any conclusions can be drawn about the efficacy of this therapy. A blinded evaluation is essential because of the recognized tendency for those changes in HBV markers which have been attributed to interferon and Ara-A therapy to occur either spontaneously in the course of CAH-B or perhaps in response to withdrawal of immunosuppressive therapy.

The cost of such therapy may be reduced greatly by the anticipated availability of interferon synthesized by bacteria which have been programed for this function by recombinant DNA technology. This prospect is made even more promising by the advent of "hybrid" interferons in a variety of molecular forms and activities produced by the introduction of segments of different human interferon genes into a single bacterial strain (C. Weissman, Biogen, Switzerland; D. Goedell, Genentech, California).

PREVENTION OF VIRAL HEPATITIS

IMMUNOGLOBUIN PROPHYLAXIS

Hepatitis A

Ordinary gamma globulin (Immune Serum Globulin: ISG) has long been recognized as highly effective in preventing clinically evident AVH-A when administered within two weeks of exposure. It has been suggested that the standard ISG dose of 0.02 ml/kg body weight for hepatitis A prevention be increased threefold on the grounds that with the declining prevalence of hepatitis A infection in this country in recent decades the anti-HAV titer in ISG may be falling (43). But the most up-to-date information available indicates that mean anti-HAV levels in ISG are actually higher now than ever before, apparently because of increasing use of paid donor plasma for preparation of this product (44). Considering this, and the fact that most physicians simply administer 2 ml of ISG to adults, which amounts to about 0.03 ml/kg body weight for the average adult, there would appear to be no reason to increase this dose.

ISG is recommended only for household contacts of person with known or suspected AVH-A, to be given as a single im injection no later than two weeks after exposure. For pre-exposure prophylaxis, such as in preparation for travel to countries where AVH-A is endemic, larger doses (0.05 ml/kg) are given every four to six months (43). The larger dose provides longer lasting protection.

Hepatitis B

There is convincing evidence that gamma globulin prepared from donor plasma units with high-titer anti-HBS activity (HBIG) is at least partially effective in preventing clinically overt hepatitis B following a potentially infecting exposure. In some cases active infection apparently occurs but its clinical expression is prevented by HBIG and permanent "passive-active" immunity results. When clinical hepatitis B develops despite HBIG injection, its incubation period may be extended to 9 months or more.

HBIG is very expensive. The cost of an average adult dose (0.06 ml/kg at $30.00/ml) is about $120. Considering this cost and the fact that essentially all commercial lots of ordinary gamma globulin (ISG) currently contain anti-HBS, at least in low titer (45), it is unfortunate that there is still uncertainty about the ability of ISG to prevent HBV infection. In two major studies which appeared to show such protection (46, 47), it was later demonstrated that the gamma globulin lots employed both actually contained HBsAg and lacked any detectable anti-HBs (48, 49). The HBsAg in these ISG lots was apparently non-infectious but it served, in effect, as a hepatitis B vaccine, causing the development of persisting anti-HBs in some inoculated persons. This effect could be distinguished from an occult infection (as seems to occur in some persons given HBIG) by the failure of ISG recipients to develop anti-HBc. The two ISG lots used in these studies were prepared before HBsAg screening of plasma used in the production of ISG was practiced. No lots of ISG manufactured since 1973 have been found to contain HBsAg (45) and so this felcitous "vaccination" reponse to ISG can no longer be expected.

HBIG may also offer some protection when administered prior to exposure as certain studies of hemodialysis workers have shown (50, 51). This has not been

recommended as routine procedure in this country because of the high cost of HBIG. HBV vaccine administration will be a better solution to this problem.

Currently the clearest indications for use of HBIG (0.06 ml/kg body weight) are; (1) for persons with parenteral or "mucosal" exposures to HBsAg-positive patients (serum or saliva in eyes, nose, or mouth; two injections are given, one month apart.) (2) For spouses or other regular sexual contacts of persons with acute HBV infection (one injection). (3) For infants born to HBsAg positive women. In the latter case, HBIG is administered immediately after birth and the injections repeated monthly for six months.

For each of these types of exposure there is good evidence that the risk of HBV transmission is much less if the HBsAg-positive subject is also anti-HBe positive, but since some small risks remains even in that case, their exposed contacts should receive HBIG nevertheless.

The risk of vertical transmission of HBV infection, which occurs at the time of parturtion, is so clearly reduced by prompt and repeat and HBIG administration to

Table 4

HBIG PROPHYLAXIS:
INFANTS BORN TO HBsAg-CARRIER MOTHERS
(Reesink, et al: Ref 65)

e-status of Mother	Treatment of Infant			
	HBIG		None	
	HBsAg		HBsAg	
	Positive	Negative	Postive	Negative
HBeAG	0	9	5	2*
Anti-HBe	0	19	1	10
Negative	0	3	0	3
Total	0	31	6	15**

p - 0.01
** p - 0.005

the infant (Table 4), and the consequences of the infection to the infant are serious enough, in the writer's opinion, to justify the routine HBsAg screening of all pregnant women during the third trimester.

NANB Hepatitis

Certain data derived from controlled studies of immunoglobulin prophylaxis of transfusion-associated hepatitis have suggested that ISG may reduce the risk of

NANB hepatitis (52, 53). Both studies showed that, while the frequency of anicteric post-transfusion hepatitis (PTH) was unaffected by large doses of ISG, icteric illness was reduced in frequency. Perhaps even more importantly, one of the studies (52) indicated that ISG recipients developed chronic hepatitis after acute PTH significantly less often than the control subjects. The authors of that study expressed the opinion that ISG prophylaxis should be given to recepients of a multiunit transfusions, but this has not become common practice.

Kikuchi et al in Japan have reported that gamma globulin modified for intravenous administration (Venoglobulin) when added directly to donor blood (250 mg/unit) before transfusion reduced the incidence of NANB-PTH from 13% to 5% (54).

Firm guidelines for ISG prophylaxis of NANB hepatitis are not yet available, but our practice at the Dallas VA Hospital has been in accord with the recommendations of Seeff (55); recognizing that the NANB viruses prevalent in this country are transmitted by the same routes as HBV, he advocates the use of ISG prophylaxis (0.06 ml/kg body weight) for the same three situations discussed above for the use of HBIG, where the exposure has been to patients with presumed NANB virus infection.

Prevention of NANB Post-Transfusion Hepatitis by Screening Blood Donors for Alanine Aminotransferase (ALT; SGPT) Elevation

In view of the frequent progression of acute NANB hepatitis to chronic hepatitis, and the recognition that this chronic infection is accompanied by a persistent viremia, it is reasonable to expect that some NANB-V carriers might be recognized among blood donors by virtue of an elevated serum ALT level. Although no specific assay for the NANB virus is generally available, observation of transfused patients for evidence of hepatitis serves, in effect, as a "biologic assay" for these viruses in donor blood.

Two recent studies have clearly demonstrated an association between donor blood ALT levels and the risks of hepatitis to recepients to that blood (56, 57).

The larger Transfusion-Transmitted Viruses (TTV) study was a cooperative effort among 4 large centers in this country (46). ALT levels were determined for samples of all blood units transfused to 1,513 patients who were then observed

Table 5 Relation between Highest ALT Level of Donor and the Incidence of Non-A,non-B Post-Transfusion Hepatitis among 1513 Recipients.

Highest ALT Level of Donor	No. of Recipients	Average No. of Units Transfused	Recipients with Non-A,non-B Hepatitis		
			Total No.	No./ 100 Recipients	No./ 1000 Units
IU					
1–14	531	2.7	24	5	17
15–29	584	4.0	37	6	16
30–44	238	4.7	35	15	32
45–59	76	4.8	22	29	60
60–284	84	4.5	38	45	101

Table 6 Relation between ALT Level of Donor and Incidence of Non-A,non-B Post-Transfusion Hepatitis among 275 Recipients of Single Units.

ALT Level of Donor	No. of Recipients	Recipients with Non-A,non-B Hepatitis
IU		*no. (per cent)*
1–14	167	7 (4)
15–29	72	5 (7)
30–44	24	2 (8)
45–59	6	2 (33)
60–284	6	3 (50)

for the development of hepatitis. The post-transfusion hepatitis rate increased progressively from 5% of patients receiving only donor blood with ALT levels below 15 iu to 45% for persons receiving at least one unit whose ALT levels exceeded 60 iu (Table 5). The figures were essentially the same for single unit recipients (Table 6).

The data from a smaller study conducted by the NIH Clinical Center Blood Bank (57) were quite similar to those of the TTV study. Viewing the combined data, it was estimated that it might be possible to prevent 20 to 30% of NANB hepatitis cases currently associated with blood transfusion. This would require the rejection of 1.6 to 3.0% of donated blood.

Before ALT screening can be advocated as a part of routine donor blood processing many experts feel that the predicted benefits should be confirmed in a prospective controlled trial. There is some doubt, however, that medicolegal pressures will allow time for such an investigation. Before embarking on routine ALT screening it will be necessary to settle a number of additional questions concerning the standardization of the enzyme assay, the exact ALT "cutoff" level for donor exclusion, the handling of excluded donors, etc.

ACTIVE IMMUNIZATION AGAINST VIRAL HEPATITIS

Hepatitis A

Whether or not administration of a hepatitis A vaccine to the general population would be desirable, its use clearly would be of value in certain groups such as military personnel, residents and employees of custodial institutions, etc.

The prospect for the development of an attenuated ~~lot~~ live virus vaccine is now a very real one since HAV has been grown in tissue culture and has shown evidence of diminished virulence after serial passage.

Hepatitis B

Krugman demonstrated the practical possibility of a hepatitis B vaccine a decade ago (59). He showed that persons inoculated with heat-inactivated MS-2 serum containing HBV responded by developing anti-HBs, (usually) without infection. These persons displayed partial immunity to later challenge with infectious MS-2 serum.

Since that time a number of vaccines have been developed and all have been shown in chimpanzee studies, and some in human studies, to be non-infectious, antigenic and protective against HBV infection. Most evidence suggests that vaccine prepared from a single strain of HBV protects patients against infections with all other strains. Presumably this is attributable to the production of some antibodies reacting with the "a-antigens" common to all HBV strains.

The most carefully studied HBV vaccine is that developed by Hilleman and associates at Merck, Sharp, and Dohme (60). Like several of the other hepatitis B vaccines, the Merck vaccine is prepared by purification of 22 nm spherules from HBsAg-positive human serum. Since the purification procedure excludes the infectious virion (Dane particles) this preparation would not be expected to

cause infection, but it is formalin treated, nevertheless, to further reduce its infectious risk.

Results of the first large scale clinical trial of the Merck vaccine have been reported by Szmuness et al (61). In this controlled, double-blind trial 1,083 homosexual men known to be at high risk of HBV infection were administered either 3 doses of vaccine or of a placebo at time zero, one month and six months. The participants in the study were all considered susceptible to HBV infection because their serum lacked HBsAg, anti-HBs and anti-HBc. Despite this, however, 2.3% of vaccinees developed anti-HBs very soon after the first vaccine injection, this anamnestic response apparently reflecting pre-existing "occult" immunity to HBV.

After the third injection. 96% of vaccine recipients had developed anti-HBs, usually in high titer (Fig. 3). No HBsAg-positive event, with or without hepatitis occurred among subjects who received all three vaccine injections and

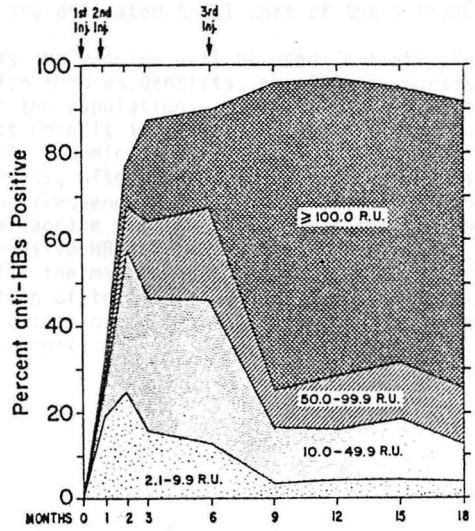

FIG. 3 Antibody (Anti-HBs) Responses and Distribution of Ratio Unit Value (RU) in Vaccine Recipients by Months after First Injection.

The cross-hatched area denotes the proportion of the antibody-positive subjects whose anti-HBs levels were at least 100.0 RU; diagonal lines, 50.0-99.9 RU; dense stippling. 10.0-49.9 RU; and light stippling, 2.1-9.9RU.

(Fig reproduced from Szmuness W et al: N Engl J Med 303:833, 1980.)

developed anti-HBs; the two subjects who became HBsAg positive after receiving all three vaccine injections were among the 4% who had failed to develop anti-HBs in response to the vaccine. Interestingly, after the HBsAg antigenemia had disappeared, these two persons finally did develop anti-HBs, apparently in response to the actual HBV infection.

This study provided evidence that the vaccine is partially protective even when given after exposure. When this finding is coupled with the observation that the active anti-HBs reponse to HBV vaccination is unaffected by HBIG given at the same time (62) the hope is offered for more complete protection against HBV infection after the kinds of exposure which now determine the need for HBIG alone.

The most important question still to be answered about the vaccine is how enduring its effects will be, and how often booster injections will be necessary to sustain immunity. There is little prospect for a live virus hepatitis B vaccine in the near future.

The FDA is expected to approve the Merck vaccine for commercial distribution before July 1982. The estimated total cost of the 3 injections is almost $100.

In Western countries the vaccine will be used initially for persons at highest risk of HBV infection such as dentists, physicians, nurses, i.e., a relatively small percentage of the population. However, such a vaccine would appear to promise the greatest benefit in those many areas of the world where chronic HBV infections are highly endemic in the general population. In such areas it is evident that this virus, often contracted at birth, causes hepatocellular carcinoma with alarming frequency. Unfortunately, many of these countries are poor and the cost of the vaccine will be well beyond their resources. But there is hope for a less expensive HBV vaccine within a few years. The region within the HBV genome coding for the major HBsAg polypeptide has been determined (63) and successful integration of this gene segment into replicating bacterial DNA (64) raises the hope that this could provide the means for producing hepatitis B vaccine at a much lower cost.

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