

## Non-A, Non-B Hepatitis

ROBERT H. PURCELL,<sup>1</sup> HARVEY J. ALTER,  
AND JULES L. DIENSTAG

*The Laboratory of Infectious Diseases, National Institute of Allergy  
and Infectious Diseases, and the Clinical Center Blood Bank,  
National Institutes of Health, Bethesda, Maryland 20014*

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### INTRODUCTION

The concept that human viral hepatitis was caused by no more than two etiologic agents stemmed from early volunteer studies in which two epidemiologically distinct types of hepatitis were transmitted to humans (1). These experimentally transmitted diseases closely resembled two forms of naturally occurring viral hepatitis that could be distinguished on epidemiologic grounds. One form was highly infectious, spread by the fecal oral route, with a relatively short incubation period of 2 to 6 weeks ("infectious hepatitis" or hepatitis type A); the other usually occurred following parenteral inoculations and was characterized by a long incubation period (approximately 6 weeks to 6 months), little, if any, person-to-person spread, and lack of association with epidemics except where multiple inoculations with the same contaminated syringe had occurred ("serum hepatitis" or hepatitis type B).

Early epidemiologic studies failed to provide evidence for more than two viral hepatitis agents (1). However, the possibility of other human hepatitis viruses was suggested by the finding of hepatitis cases with an incubation period intermediate between that of hepatitis A and B viruses (2). More recently, multiple cases of hepatitis occurring among illicit drug users suggested that three or more etiologic agents might exist (2).

With the discovery of hepatitis B surface antigen (HB<sub>s</sub>Ag) and its association specifically with type B hepatitis (3, 4), a method to reevaluate accepted beliefs about type B hepatitis became available. Some surprises emerged. Among these was the finding that a significant proportion of "sporadic" hepatitis not acquired by demonstrable percutaneous exposure was, in reality, associated with HB<sub>s</sub>Ag. Furthermore, epidemiologic surveys for HB<sub>s</sub>Ag and antibody to HB<sub>s</sub>Ag (anti-HB<sub>s</sub>) provided serologic evidence of exposure to hepatitis B virus (HBV) among a proportion of affected individuals whose mode of infection was incompatible with the concept of strictly percutaneous spread of the virus (5). Even more interesting was the finding that a significant proportion of transfusion-associated hepatitis could not be related serologically to hepatitis B virus infection (6, 7). It was thought that such "non-B" hepatitis was undiagnosed type B infection or, more likely, percutaneously transmitted type A hepatitis. However, epidemiologic studies as far back as 1962 strongly suggested that the latter was not the case: The incubation period of transfusion-associated hepatitis defined a unimodal curve with its peak 45 to 49 days after exposure, strongly suggesting that short incubation period type A hepatitis was not an important cause of illness in blood recipients (8). Similar conclusions were reached in a recent study of transfusion-associated hepatitis (9).

<sup>1</sup>Reprint requests to: Dr. Robert H. Purcell, NIH, Building 7, Room 202 Bethesda, Md. 20014.

## HEPATITIS

*Nomenclature*

The designation of "infectious hepatitis" and "serum hepatitis" for the two recognized forms of viral hepatitis imbued them with erroneous epidemiologic qualities. For this reason, the less connotative names "hepatitis, type A" and "hepatitis, type B" (10, 11) were revived, but the concept of two and only two types of hepatitis survived, and all hepatitis not identifiable as type B was defined by exclusion as type A. To avoid such an assumption, Blumberg suggested that viral hepatitis should be classified as being Australia antigen (HB<sub>s</sub>Ag) positive or negative (12). Currently available tests for markers of hepatitis B virus infection (13) are sufficiently sensitive to detect almost all hepatitis B virus infections; thus it seems reasonable, in the absence of definitive evidence for hepatitis A virus infection, to modify Blumberg's proposal to classify patients with any serologic evidence of primary exposure to hepatitis B antigens as type B and those with no serologic evidence of exposure as type non-B.

The development of sensitive tests for indicators of hepatitis A virus infection (hepatitis A antigen and antibody) now makes it possible to divide non-B hepatitis into type A hepatitis and non-A, non-B hepatitis (14–20). Although the term "type C hepatitis" has been suggested for the latter disease (9, 21), there is evidence that non-A, non-B hepatitis may be caused by more than one agent (see below), and we believe it wiser, therefore, to use the less mellifluous but more accurate designation type non-A, non-B hepatitis. The term type C hepatitis should be reserved until type non-A, non-B hepatitis can be defined serologically.

*The Role of Identifiable Agents in Non-A, Non-B Hepatitis*

Both cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are known to cause hepatitis as part of the generalized infection characteristic of these herpesviruses (22, 23). Epstein-Barr virus appears to play a minor role in type non-B hepatitis, as judged by the infrequency of serologic evidence of associated infection with this virus (6, 24–28). The role of CMV in type non-B hepatitis is more difficult to evaluate because of the presence of multiple, partially related serotypes of the virus and a demonstration of variation in anti-CMV antibody levels in normal individuals followed longitudinally (29, 30). Convincing evidence for an etiologic role of CMV in some cases of hepatitis associated with transfusion or immunosuppression has been presented (23, 31, 32), but most cases of type non-B hepatitis lack such evidence, and serologic evidence of recent CMV infection can be found as frequently in transfused patients who develop hepatitis as in those who do not (6, 9, 33, 34). Thus, the importance of CMV in the etiology of type non-B transfusion-associated hepatitis is difficult to evaluate but is probably not great.

*Epidemiology*

Type non-A, non-B hepatitis was first identified in transfused patients and was distinguished from type A hepatitis on both epidemiologic and serologic grounds (9, 24–26, 35, 36). Although an etiologic agent could not be ascribed to over half of the cases of transfusion-associated hepatitis detected in early studies (6), it was assumed that many of these were caused by HBV but could not be diagnosed because of the relative insensitivity of tests for HB<sub>s</sub>Ag and anti-HB<sub>s</sub> available at that time. However, few additional HBV infections were identified when more sensitive serologic tests were applied to serial samples obtained from these longitudinally

followed patients (Purcell, R. H., unpublished). Furthermore, the combined impact of HB<sub>s</sub>Ag-screening programs and the curtailment of the use of commercially derived blood has resulted in a smaller proportion of transfusion-associated hepatitis caused by HBV. At present, as much as 90% of such hepatitis appears to be type non-A, non-B (26, 37, 38). Hepatitis A virus, serologically related to the MS-1 strain of virus described by Krugman *et al.* (39), appears to be responsible for most episodes of epidemic hepatitis, including water-borne and food-borne outbreaks, explosive epidemics occurring within institutions and families, shellfish-associated hepatitis, and hepatitis among handlers of nonhuman primates. The distribution of this virus is probably worldwide. In contrast, non-B "sporadic" hepatitis not associated with the epidemiologic settings described above appears to be caused by both HAV and non-A, non-B agents.

Although data are as yet limited, type non-A, non-B hepatitis has been detected throughout the world, including the United States (25, 40), Japan (Moritsugu, Y., personal communication), Costa Rica (41), and, possibly, Australia (Gust, I. D., *et al.*, in preparation), Great Britain (42) and Germany (43). Within the United States, type non-A, non-B hepatitis has been detected wherever it has been sought; in one study, over half of the non-B sporadic hepatitis observed was found to be type non-A, non-B (Mosely, J. W., *et al.*, in preparation). It therefore seems likely that a significant but undetermined proportion of the greater than 50,000 cases of viral hepatitis reported annually in the United States is type non-A, non-B hepatitis.

As with type B hepatitis, type non-A, non-B hepatitis occurs significantly more frequently following transfusion of commercially derived blood than following receipt of blood derived from volunteer sources. Thus, type non-A, non-B disease (or non-B hepatitis presumed to be non-A) has been found to occur five to ten times more frequently following transfusion of the former than following transfusion of the latter (9, 37, 38). Despite increasing use of voluntarily donated blood and application of the most sensitive techniques for screening blood for HB<sub>s</sub>Ag, type non-A, non-B hepatitis is still occurring at a rate of three to six cases/1000 units of blood transfused (26, 37, 38).

The epidemiology of type non-A, non-B hepatitis resembles more closely that of type B hepatitis than that of type A disease. Thus, type non-A, non-B hepatitis occurs commonly following parenteral exposure to blood or illicit drugs, appears to be endemic in many populations but is not readily spread from person to person, occurs more commonly among those of low socioeconomic status such as commercial blood donors, and, on the basis of the observed rate of disease following transfusion, must result in an infectious carrier state.

#### *Clinical Characteristics*

The average non-A, non-B hepatitis case appears to differ clinically in several small but significant ways from type B illness, but, as with the latter, the clinical expression of the former is so variable that a diagnosis on clinical grounds alone cannot be made reliably in individual cases.

On the basis of limited studies, the average incubation period of type non-A, non-B hepatitis appears to be 6 to 7 weeks shorter than that of type B hepatitis (24-26), but the extreme variability of the incubation period makes its determination of little value for diagnosis (Table 1). Interestingly, these incubation periods for serologically confirmed type non-A, non-B and type B hepatitis are very similar to those reported by Mosely (36) for hepatitis occurring after administration of whole blood only (9.9

TABLE 1  
Transfusion-Associated Hepatitis: Incubation Period

Type of hepatitis	Number	Mean incubation period (weeks) <sup>a</sup>	Range
B <sup>b</sup>	18	14.4	4-25
Non-A, non-B	22	7.3	2-22

<sup>a</sup>From transfusion to first elevated SGPT value.

<sup>b</sup>HB<sub>s</sub>Ag positive.

weeks) and blood products only (14.6 weeks) during the period 1961-1965, prior to widespread testing for HB<sub>s</sub>Ag, and provide additional suggestive evidence that over half of the transfusion-associated hepatitis reported at that time was not caused by HBV (6). Others have found less difference in incubation periods between type B hepatitis and non-B cases (9, 37, 38).

Type B hepatitis is more acutely severe than type non-A, non-B disease, resulting in a higher proportion of icteric cases and a higher mean maximum SGPT value (24-26) (Table 2). Again, the range of maximum SGPT values is too broad to be of value for diagnosis. A greater severity of type B hepatitis when compared to non-B disease has also been noted in previous studies (9, 37, 38).

Although type non-A, non-B hepatitis is associated with less severe acute illness than type B disease, as judged by frequency of jaundice and magnitude of SGPT elevations, the long-term prognosis for the two diseases may be similar. Thus, elevation of transaminase values persisting for 6 or more months has been observed more frequently following non-A, non-B disease than following type B hepatitis (Table 3). Others have reported similar results (37). Transaminase elevations have been documented for several years in some patients. Three such patients at the NIH underwent liver biopsy; two had histopathologic changes in the liver compatible with chronic active hepatitis, and the other was diagnosed as having chronic persistent hepatitis (44; Alter, H. J., *et al.*, unpublished). Thus, chronic non-A, non-B hepatitis is not necessarily a benign infection and may be the cause of a significant proportion of chronic hepatitis not identifiable as type B disease.

An interesting feature of some cases of chronic non-A, non-B hepatitis is the episodic nature of transaminase elevations; periods of transaminase elevation alternate with periods of normal or near normal transaminase activity. Such a cyclic

TABLE 2  
Transfusion-Associated Hepatitis: Severity

Type of hepatitis	Number	Number icteric	Mean maximum SGPT value	Range
B	19	12 (63%)	768	157-2050
Non-A, non-B	22	6 (27%)	395	120-1476

TABLE 3  
Transfusion-Associated Hepatitis: Duration of Illness

Type of hepatitis	Number	Number with SGPT elevation persisting > 6 months
B	19	3 (6%)
Non-A, non-B	21	7 (33%)

pattern of hepatitis activity appears to occur more frequently in the patient with chronic (or persistent) non-A, non-B hepatitis than in the individual chronically ill with type B disease and care must be exercised that these repeating episodes of hepatitis are not interpreted as representing separate and distinct bouts of acute hepatitis.

*Attempts to Identify the Agent (or Agents) of Non-A, Non-B Hepatitis*

Several approaches to the identification of agents or antigens associated with non-A, non-B hepatitis are being pursued. Among these is a search for an antigen analogous to hepatitis A and B antigens but specific for non-A, non-B hepatitis. In these experiments, acute phase serum and plasma from patients with non-A, non-B hepatitis are being examined by solid phase radioimmunoassay and immune electron microscopy techniques for unique antigens. In addition, chimpanzees and other species of nonhuman primates are being inoculated with acute phase serum or plasma from patients with non-A, non-B hepatitis. To date, none of these approaches has yielded unequivocally positive results, but the search continues for the agent of non-A, non-B hepatitis.

Failure to demonstrate non-A, non-B hepatitis in chimpanzees may be explained by an insusceptibility of this species to the virus; susceptibility resulting in inapparent (undetected) infection; or presence of immunity resulting from a prior naturally acquired infection. However, the failure, to date, to detect an antigen specific for type non-A, non-B hepatitis or to transmit an agent to chimpanzees, a species known to be susceptible to both hepatitis A and B viruses, also suggests that the agent of this disease may be present in relatively low titer in acute phase serum or that it is relatively labile and destroyed by freezing, storage at  $-70^{\circ}\text{C}$  and/or subsequent thawing. Alternatively, the agent may be cell associated and therefore inseparably bound to one of the cellular components of blood. Such cell association is a common characteristic of many herpesviruses such as CMV and varicella zoster virus (45). That one or more of these latter predictions is correct is suggested by the observation that non-A, non-B hepatitis occurs infrequently, if at all, following parenteral administration of *processed* blood products such as clotting factors, a finding anticipated by the epidemiologic study of Mosely (36). However, additional studies must be carried out to determine if the largely anicteric disease associated with non-A, non-B hepatitis has been overlooked in previous studies of hepatitis following administration of these components.

One transmissible agent that causes hepatitis in marmosets has been proposed as a candidate for non-A, non-B hepatitis virus. In 1967, Deinhardt *et al.* reported the isolation and serial transmission in marmosets of an agent thought to be hepatitis A virus (46). Parks and Melnick subsequently cast doubt on the authenticity of this agent, named the GB agent after the surgeon from whom it was isolated, and suggested that this virus was a contaminant, possibly a latent virus of marmosets, and not related to human hepatitis (47, 48). Subsequent studies by Deinhardt and his group resulted in the transmission of a bona fide hepatitis A virus, the MS-1 strain, to marmosets and the demonstration that this hepatitis A virus was distinct from the GB agent (49). Paired serum samples from the surgeon from whom the GB agent was recovered have been studied for serologic evidence of hepatitis A and B virus infection; evidence for recent infection with either of these viruses could not be detected (Dienstag, J. L., and Deinhardt, F., unpublished). Therefore, GB may have had non-A, non-B hepatitis. Deinhardt has suggested that the GB agent is, in fact, a non-A, non-B hepatitis agent (21), but the inability to perform serologic tests with the GB agent (other than cross-challenge experiments in marmosets) has made it impossible

to determine the significance, if any, of the GB agent in the etiology of non-A, non-B hepatitis.

#### *Evidence for More than One Non-A, Non-B Hepatitis Agent*

Mosley recently reported the documentation of three separate bouts of hepatitis in six patients and four distinct bouts in an additional five patients longitudinally followed in a study of hepatitis in Los Angeles and cited other reports of multiple bouts of hepatitis (2). Serologic analysis of these multiple bouts of hepatitis (Mosely, J. W., *et al.*, submitted for publication) has revealed that as many as two and three separate bouts of hepatitis in one individual were not identifiable as types A or B and were therefore probably type non-A, non-B hepatitis. These bouts did not appear to be instances of recurrent chronic type non-A, non-B hepatitis, as described above, for liver biopsy revealed each of them to be associated with histopathologic evidence of acute hepatitis. Furthermore, there was no serologic evidence that any of these bouts of hepatitis were caused by EBV or CMV. Thus, there is seroepidemiologic evidence that two or more agents may be responsible for non-A, non-B hepatitis, but more definitive studies must await the discovery of serologic markers for non-A, non-B disease.

#### *Prevention of Type Non-A, Non-B Hepatitis*

Numerous studies of the efficacy of immune serum globulin for prevention of transfusion-associated hepatitis have yielded equivocal results (50). Only one study to date has carefully segregated such hepatitis into type B and non-B (presumably non-A, non-B) (38). In this study immune serum globulin afforded minimal, if any, protection in preventing non-A, non-B hepatitis when compared to an albumin placebo.

At present the most effective means of preventing transfusion-associated non-A, non-B hepatitis is the substitution of blood derived from volunteer sources for commercial blood. The exclusive use of volunteer blood has been shown to reduce markedly (but not eliminate) the incidence of both type B hepatitis and type non-A, non-B disease (37, 38, 44).

### CONCLUSIONS

There is evidence that at least one additional agent is etiologically responsible for human viral hepatitis; hepatitis caused by this agent (or agents) is unrelated to hepatitis caused by hepatitis A or B viruses. The epidemiology of such non-A, non-B hepatitis resembles that of type B hepatitis more than that of type A disease. Type non-A, non-B hepatitis can progress to a chronic state. Attempts to identify an antigen specific for non-A, non-B hepatitis or to transmit the disease to nonhuman primates have, to date, been unsuccessful. Such lack of success may reflect the lack of appropriate reagents or may suggest that the agent is cell associated, labile, or present in relatively low titer. The role of immune serum globulin in the prevention or modification of type non-A, non-B hepatitis is poorly defined; at present, the most effective means of diminishing the frequency of transfusion-associated non-A, non-B hepatitis is the use of blood derived solely from volunteer sources.

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