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The Dominant Role of Non-A, Non-B in the Pathogenesis of Post-transfusion Hepatitis: A Clinical Assessment

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Although clinically recognized since the time of Hippocrates, viral hepatitis has been a continually enigmatic disease with each new insight raising the spectre of a new complexity. Despite remarkable advances in the past decade emanating from the discovery of the Australia antigen (Blumberg, Alter and Visnich, 1965) and its eventual linkage to viral hepatitis type B (Prince, 1968), there remain a multitude of unresolved issues regarding aetiology, routes of transmission, host-virus interactions, chronic carrier states, relationship to chronic liver disease and hepatocellular carcinoma, methods of propagation and modes of treatment. The most recent addition to this compendium of unknowns is the classification of an increasing number of viral hepatitis cases into the category 'non-A, non-B'. Despite the absence of a confirmed infectious particle or a specific diagnostic test, there is increasing circumstantial and direct evidence that the agent of non-A, non-B hepatitis is by far the major cause of transfusion-associated hepatitis and one of the leading causes of chronic hepatitis, particularly chronic active hepatitis. The remainder of this chapter will cite the evidence for the existence of a non-A, non-B agent, document its relationship to post-transfusion hepatitis, present the pattern of its clinical presentation, support its frequent relationship to chronic liver disease and demonstrate that it is due to a transmissible agent which, though presumed to be a virus, has not been established as such. Lastly, this review will attempt to present the current direction of research to better define this increasingly recognized disease entity.

HISTORICAL PERSPECTIVE

Classically, viral hepatitis has been subdivided into two clinically and, more recently, serologically distinct entities. One form was characterized by a short incubation period and an explosively epidemic transmission pattern which resulted in large clusters of cases which generally could be traced to a

common source. This 'infectious' hepatitis was shown to be transmitted primarily by the faecal-oral route and was distinguished from 'serum' hepatitis, which had a longer incubation period and more indolent transmission pattern generally thought to require percutaneous inoculation of blood or a blood product. The existence of two distinct forms of viral hepatitis was further confirmed by the studies of Krugman, Giles and Hammond (1967), who demonstrated under controlled conditions that two epidemiological forms of hepatitis (MS-1 and MS-2) were endemic in an institutional setting. Of major significance in these studies was not only the clear-cut separation of these two forms of viral hepatitis by incubation period and mode of transmission, but also their immunological distinction based on cross-challenge experiments. When tests for hepatitis B surface antigen (HBsAg, Australia antigen) became available, the immunological distinction of these two entities was further amplified. Short incubation, MS-1 disease (infectious hepatitis) was consistently HBsAg negative, whereas long incubation, MS-2 disease (serum hepatitis) was HBsAg positive. The serological differention of these two forms of viral hepatitis was further confirmed when tests for HBsAg were applied to classic epidemics of infectious hepatitis and shown to be uniformly HBsAg negative, whereas cases of post-transfusion or post-inoculation hepatitis were frequently HBsAg-positive.

The above suggested a very straightforward pattern for viral hepatitis. One form was highly infectious, spread by the faecal-oral route, had a short incubation period and was HBsAg negative. The other form was not readily spread from person to person, was transmitted primarily by parenteral routes, had a long incubation period and was HBsAg positive.

Several observations, however, complicated this simple interpretation. First, using HBsAg and its corresponding antibody (anti-HBs) as markers, it was shown that many individuals developed HBsAg-positive, serum hepatitis and yet had no known exposure to any blood product. 'Serum' hepatitis appeared to be a misnomer and it seemed appropriate to revert to an earlier nomenclature in which infectious hepatitis was referred to as hepatitis A and serum hepatitis as hepatitis B. Such designation acknowledges the distinctions between these diseases, but does not misleadingly restrict the mode of transmission. Second, it was noted by Mosley (1975) that some cases of hepatitis had an incubation period intermediate between that of classic type A and type B hepatitis, suggesting the possibility of a third human hepatitis virus. Third, and most significant, many cases of post-transfusion hepatitis could not be serologically or epidemiologically related to either the type A or type B viruses. It was from the evaluation of these cases that the concept of non-A, non-B hepatitis evolved and this will be discussed in detail in the section on post-transfusion hepatitis below.

DIAGNOSIS AND NOMENCLATURE

In the presence of an elevated serum transaminase, with or without other symptoms, signs or biochemical evidence of liver dysfunction, the diagnosis of non-A, non-B hepatitis depends upon the clinical exclusion of other causes

of hepatocellular injury and the serological exclusion of other known hepatitis viruses. There are no pathognomonic clinical features and no specific serological tests by which the diagnosis can be established. It is thus, as its name implies, a diagnosis of exclusion. Although extremely awkward, the terminology 'non-A, non-B hepatitis' is retained in this chapter because it states the facts as we currently know them and does not presume knowledge which cannot be documented. It is tempting for the sake of simplicity to call this hepatitis 'C' but, in point of fact, we do not know if non-A, non-B hepatitis is due to a single virus or to a series of viruses and indeed, although a viral aetiology is highly probable, even this has not been proved. The adoption of a more specific nomenclature must await the serological definition of non-A, non-B hepatitis.

INCIDENCE OF NON-A, NON-B FOLLOWING TRANSFUSION

The introduction of tests for HBsAg made it possible for the first time to serologically define cases of post-transfusion hepatitis (PTH). Although this antigen was a marker for 'serum' hepatitis, early studies (Purcell et al, 1971; Gocke, 1972) indicated that only 25 per cent of PTH cases were HBsAg positive. This surprisingly low incidence of type B hepatitis in the transfusion setting was thought to reflect the insensitivity of tests for HBsAg then in use and the fact that a significant proportion of PTH was due to the hepatitis A virus.

For the most part, each of these assumptions was in error. Although insensitive serological methods did account for the failure to diagnose some cases of type B PTH, the failure rate was unexpectedly small. Even when very sensitive radioimmunoassays for both hepatitis B surface antigen and antibody were applied, no more than one third of PTH cases could be attributed to the hepatitis B virus. It was then assumed that a large proportion of post-transfusion hepatitis cases were due to the hepatitis A virus, but many factors militated against this assumption. Allen and Sayman (1962) demonstrated that the incubation period of PTH defined a unimodal curve with a peak incidence at 45 to 49 days after exposure. This relatively long incubation period was not consistent with that produced by the hepatitis A virus and suggested an alternative aetiology. Mosley (1975) reiterated this point in his review of viral hepatitis and suggested that the distribution curve for the onset of PTH was intermediate between that of classic type A and type B hepatitis, raising the possibility of an additional human hepatitis virus playing a major role in this disease. Prince et al (1974), in a controlled study testing the efficacy of gamma globulin for the prevention of PTH, demonstrated that 71 per cent of observed hepatitis cases were serologically unrelated to the hepatitis B virus. Epidemiological observations suggested that these non-B hepatitis cases were unlikely to represent hepatitis A because of their long incubation period, because of the lack of evidence for intrafamilial transmission, and because of the complete failure of gamma globulin to abrogate this disease. It was not until 1975, however, that it was conclusively documented that the hepatitis A virus did not play a significant role in the causation of PTH. Applying the technique of immune electron

microscopy to hepatitis cases detected in prospective studies at the National Institutes of Health (NIH), Feinstone et al (1975) reported that not one of 22 cases of non-B PTH demonstrated serological evidence of recent exposure to the hepatitis A virus. In addition, none of these 22 cases of non-B hepatitis demonstrated antibody seroconversion to other known human hepatitis viruses, namely the cytomegalovirus (CMV) and the Epstein-Barr virus (EBV). This strongly implicated a previously unrecognized human hepatitis virus which was designated non-A, non-B. Alter et al (1975) then demonstrated that, in recipients of volunteer donor blood screened for HBsAg by radioimmunoassay, almost 90 per cent of the observed hepatitis could be classified as non-A, non-B.

Table 1 depicts representative incidence figures for non-B PTH in prospectively followed patients and relates this to donor source and HBsAg testing procedure. The proportion of hepatitis cases classified as non-B ranged from 71 to 97 per cent. As would be anticipated, the proportion of type B cases was highest in those studies in which blood donors were initially screened for HBsAg by agar gel diffusion or counterelectrophoresis and, conversely, the proportion of non-B cases was generally highest in those utilizing donors screened by RIA. In those studies (Goldfield, 1975; Aach et al, 1978; Alter et al, 1978a; Seeff et al, 1978) initially screening donors by RIA, the mean percentage of hepatitis cases classified as non-B was 92 per cent. The same percentage were also classified as non-A, non-B since, in each of these four studies, hepatitis cases were evaluated for a serological

Table 1. *Incidence of non-B hepatitis in prospective studies of post-transfusion hepatitis*

Reference	Donor source/test	No. studied	% with hepatitis	% of total hepatitis non-A, non-B
Prince et al (1974)	CV/AGD, CEP	204	25[a]	71[c]
Alter et al (1975)	V/CEP (RIA)	108	11	75 (89)[d]
Goldfield (1975)	CV/RIA	563	13	93
Knodell et al (1976)	MV/CEP (RIA)	279	17[b]	94 (96)[d]
Alter et al (1978a)	V/RIA	388	8	90
Seeff et al (1978)	CV/RIA	969	13[a]	97
Aach et al (1978)	CV/RIA	595	13	87

Abbreviations: V = volunteer blood only, CV = mixture of commercial and volunteer donors, MV = military volunteer donors, AGD = agar gel diffusion, CEP = counterelectrophoresis, RIA = radioimmunoassay.

[a]Studies done to evaluate immune serum globulin (ISG) and/or hepatitis B immune globulin (HBIG) in the prevention of post-transfusion hepatitis. Since neither globulin preparation was shown to reduce the incidence of total hepatitis or non-B hepatitis, the observed hepatitis incidence figures are considered valid representations of what would have occurred had no globulin been given.

[b]Both ISG and HBIG were claimed to have reduced the incidence of hepatitis as compared with a placebo control so that cited figures might underestimate the incidence in an untreated transfused population.

[c]Not tested for antibody to hepatitis A.

[d]Initially screened by CEP, but subsequently retested by RIA. Results in parenthesis indicate expected percentage had RIA-positive, CEP-negative donors been excluded.

response to the hepatitis A virus and no such response was demonstrated. The lowest overall incidence of PTH (8 per cent) was noted in those recipients of non-military, volunteer donor blood which had been prescreened by radioimmunoassay.

OCCURRENCE OF NON-A, NON-B WITHOUT PRIOR TRANSFUSION

Elucidation of the transmission pattern of type B hepatitis has depended upon the ability to test various populations for the presence of hepatitis B antigens and antibodies. The absence of such serological markers in non-A, non-B hepatitis severely restricts epidemiological investigation of this disease. Nonetheless, its frequent occurrence in the transfusion setting has been clearly documented as noted above, and there is now increasing evidence that non-A, non-B also occurs without prior percutaneous exposure. Villarejos et al (1975) studied 103 patients with 'sporadic' hepatitis occurring in an endemic zone of Costa Rica. Twelve cases were encountered in which both type A and type B hepatitis could be excluded on the basis of serological testing. These patients had not been transfused or had other needlestick exposure and available evidence suggested person to person transmission of non-A, non-B hepatitis. Dienstag et al (1977) investigated 40 cases of non-B sporadic hepatitis requiring hospitalization in Los Angeles. Fifty per cent of these non-B cases showed serological evidence of type A infection and 50 per cent were classified as non-A, non-B. In 10 of the 20 non-A, non-B cases there was no known percutaneous exposure. Norkrans (1978) studied the probable routes of infection in 66 cases of non-A, non-B hepatitis in Sweden. In 33 per cent there was no identifiable transfusion or needlestick exposure. Müller et al (1978) reported that 19 per cent of symptomatic cases of acute hepatitis occurring in the Federal Republic of Germany were classified as non-A, non-B and that in 40 per cent of these cases there was no known transfusion or other percutaneous inoculation.

Although discussion of the modes of transmission of non-A, non-B hepatitis must be tentative until a serological marker is identified, it is probable that its transmission pattern will be very similar to type B hepatitis and very dissimilar to type A hepatitis. As with type B hepatitis, it is prevalent following transfusion or other covert or overt percutaneous exposure; it occurs in endemic form and is found in higher frequency among populations of low socioeconomic status; it is probably spread by close person to person contact (questionable salivary or venereal transmission), but is not transmitted in epidemic form; and it is associated with a chronic carrier state, as will be amplified below.

CLINICAL CHARACTERISTICS

Although recognition of non-A, non-B hepatitis has been a relatively recent event, a characteristic, though not pathognomonic, clinical pattern is beginning to emerge. This pattern has evolved primarily from prospective studies of post-transfusion hepatitis and some of the salient features are

summarized in Table 2. Comparable values for type B hepatitis are not shown, but in each of these studies type B hepatitis had a longer mean incubation period, a higher mean alanine aminotransferase (ALT, SGPT), and a greater percentage of icteric cases. Type B hepatitis thus tends to be more acutely severe than non-A, non-B. However, as indicated by the broad range of clinical presentation, in any individual case one cannot distinguish non-A, non-B hepatitis from type B hepatitis on the basis of incubation period, peak ALT or the presence or absence of icterus.

Table 2. *Clinical characteristics of non-A, non-B hepatitis*

Reference	No. with non-A, non-B hepatitis	Mean incubation period/weeks (s.e. or range)	Mean peak SGPT/IU (s.e. or range)	No. icteric
Prince et al (1974)	36	8.0 (±2.7)	259 (±131)	14 (39%)
Seeff et al (1978)	119	8.4 (2—26)	286 (62—920)	21 (18%)
Aach et al (1978)	65	6.3 (2—13)	647 (±75)	16 (25%)
Alter et al (1978a)	26	8.2 (5—20)	744 (132—2322)	8 (31%)

Although very short incubation disease (two weeks) is classified as non-A, non-B in some of these studies as well as a recent study from Japan (Shirachi et al, 1978) and although some had an incubation period as long as 26 weeks, the incubation period of non-A, non-B hepatitis, in general, describes a sharp unimodal curve with a peak onset at about eight weeks after exposure. As a rule non-A, non-B hepatitis is symptomatically mild; roughly 75 per cent of cases are anicteric and have peak ALT values less than 800 IU/l. Rarely does a patient require hospitalization. Nonetheless, in any individual case, the patient may be seriously ill with striking jaundice and markedly elevated hepatic enzymes. Another clinical characteristic of non-A, non-B hepatitis, not indicated in Table 2, is the tendency for the transaminase level to fluctuate markedly over relatively short time intervals; at other times, long intervals of normal ALT values are interspersed with distinct elevations. Indeed, because of these recurring enzyme abnormalities, it is difficult to ascertain when this disease has resolved; arbitrarily, a minimal criterion for resolution should be at least six consecutive monthly samples with normal ALT values.

In summary, acute non-A, non-B hepatitis is typically a mildly symptomatic or asymptomatic disease. Two thirds to three quarters of cases are anicteric with peak ALT values less than 800 IU/l. Individual cases may, however, be severe and for this reason cannot be clinically distinguished from type A or type B hepatitis. The vast majority of cases have their onset six to 10 weeks after exposure, but the broad range of incubation periods again does not permit distinction from either type A or type B hepatitis. Widely fluctuating enzyme patterns are common and biochemical resolution is difficult to assess. The most prominent and probably the most important feature of non-A, non-B hepatitis is the propensity for abnormal ALT values

to persist for prolonged periods and for hepatic histology to demonstrate features of chronic persistent or chronic active hepatitis (see below).

CHRONIC SEQUELAE OF NON-A, NON-B HEPATITIS

The clinical pattern of non-A, non-B hepatitis presents a striking example that anicteric hepatitis cannot be ignored and attests to the importance of prospective follow-up in recipients of blood transfusion. Although the majority of cases are anicteric and asymptomatic, up to 50 per cent of these patients have abnormal ALT values for greater than one year and, when biopsied, most show histological evidence of significant chronic liver disease. Table 3 illustrates this point in some representative studies. The study of Knodell, Conrad and Ishak (1977) represents a randomized trial to compare immune serum globulin, hepatitis B immune globulin and an albumin placebo in the prevention of PTH. Since the investigators claim a reduced progession to chronic non-A, non-B hepatitis in recipients of either globulin preparation, the frequency of chronic liver disease in Table 3 represents a minimum figure. An additional study by Koretz, Suffin and Gitnick (1976) is not included in Table 3 because the pathological diagnoses could not be tabulated on the basis of an aetiologic agent. However, of 47 patients who developed PTH, 29 (62 per cent) had an abnormal ALT for greater than 20 weeks and only nine of these 29 represented type B hepatitis. Liver biopsies were performed in 15 of the 29 patients; nine demonstrated chronic active hepatitis, two chronic persistent hepatitis, and four unresolved hepatitis. Five of the nine patients with chronic active hepatitis were asymptomatic. Cirrhosis was not seen in any biopsy and there were no fatalities.

To summarize the chronic effects of non-A, non-B hepatitis, approximately 25 to 50 per cent of patients have abnormal ALT values for at least six

Table 3. *Chronic sequelae of non-A, non-B hepatitis*

Reference	No. with non-A, non-B hepatitis	No. with abnormal ALT		Liver biopsy				
		6 months	1 year	No.	CAH	CPH	NS	Cirrhosis
Galbreath et al (1975)	29	8	8 (28%)	7	3	2	2	2[c]
Knodell, Conrad and Ishak (1977)	44	NR	10 (23%)[a]	10	8	1	0	1
Seeff et al (1978)	119	31 (26%)	12 (10%)	NR	—	—	—	—
Aach et al (1978)	65	36 (55%)[b]	NR	NR	—	—	—	—
Berman et al (1979)	26	12	12 (46%)	8	6	2	0	1[c]
		Total 25			17 (68%)	5 (20%)	2 (8%)	4 (16%)

Abbreviations: ALT = alanine aminotransferase (SGPT); CAH = chronic active hepatitis; CPH = chronic persistent hepatitis; NS = non-specific hepatitis; NR = not reported.

[a]Incidence might be affected by gamma globulin administration (see text).
[b]ALT abnormal for at least 40 weeks in all 36 cases.
[c]These represent patients with CAH who also had evidence of cirrhosis.

months and most of these for greater than one year after the onset of their disease. When biopsied, the majority of patients show evidence of chronic active hepatitis and approximately 10 per cent have histological evidence of cirrhosis. Although bridging necrosis is rarely seen, these biopsies fulfil the histological criteria for chronic active hepatitis (Scheuer and Thaler, 1977). Clinically, however, the chronic active hepatitis associated with non-A, non-B hepatitis seems to run a benign course. Patients tend to be asymptomatic or troubled only by easy fatigability. There are no associated autoimmune phenomena and tests for anti-nuclear, anti-smooth muscle and anti-mitochondial antibody are usually negative. Clinical symptoms or physical stigmata of severe chronic liver disease are generally absent. Specifically, ascites, gastrointestional haemorrhage, coma and death due to chronic liver failure have not been reported. This may reflect the relatively short duration these patients have been followed, but may also reflect that chronic non-A, non-B hepatitis is a slowly resolving rather than rapidly progressing lesion. This is supported, in part, by gradually declining transaminase values in most patients over a period of one to four years after the onset of their disease. The gradual trend toward normalization can be noted despite the aforementioned fluctuations in ALT values. Some patients with chronic active hepatitis in the NIH study (Berman et al, 1979) have demonstrated apparent biochemical resolution of their disease, but have not yet been rebiopsied to determine if this is accompanied by a parallel histological resolution.

EVIDENCE FOR A TRANSMISSIBLE AGENT AND CHRONIC CARRIER STATE

As in hepatitis B, the chimpanzee has become the primary animal model for establishing the presence of an infectious agent in non-A, non-B hepatitis. In 1978, Alter et al and Tabor et al simultaneously reported the first transmission of non-A, non-B hepatitis to the chimpanzee. In the study of Alter et al (1978b) serum or plasma from two patients with acute non-A, non-B hepatitis, from two patients with chronic non-A, non-B hepatitis and from one implicated donor were inoculated into five chimpanzees and in each case resulted in biochemical and histological evidence of viral hepatitis. In the study of Tabor and coworkers (1978), serum from a patient with chronic non-A, non-B hepatitis, whose blood appeared to transmit the disease to a nurse following accidental needlestick, was inoculated into two chimpanzees. Two additional chimpanzees were inoculated with blood from two implicated donors who had elevated serum transaminase. Each of the four chimpanzees in this study developed biochemical and histological evidence of non-B hepatitis. Hollinger et al (1978) demonstrated transmission to five chimpanzees using sera obtained from implicated donors with and without elevated transaminase values. Prince et al (1978) infected 17 chimpanzees utilizing 'preacute' phase sera from 10 patients with non-A, non-B hepatitis as the inoculum. Lastly, in a reanalysis of human volunteer studies performed in the early 1950s, Hoofnagle et al (1977) showed that one millilitre of serum from three implicated, HBsAg-negative, asymptomatic blood

donors caused icteric non-A, non-B hepatitis in from 10 to 47 per cent of recipients.

In composite, the above studies document two crucial points in the epidemiology of non-A, non-B hepatitis: (1) Non-A, non-B hepatitis is caused by a transmissible agent. Blood-borne transmission from human to human, from human to chimpanzee and from chimpanzee to chimpanzee has now been well documented. (2) There is a chronic carrier state for the non-A, non-B agent(s) and such carriers may either have evidence of chronic liver disease or be totally asymptomatic with no demonstrable abnormalities of liver function. A chronic non-A, non-B carrier state has previously been inferred from the large number of non-A, non-B PTH cases which derive from seemingly healthy donors. These chimpanzee transmission studies now firmly establish the existence of such a carrier state. Overall, the transmission pattern for non-A, non-B hepatitis appears to be very similar, if not identical, to that previously documented for type B hepatitis.

EVIDENCE FOR MORE THAN ONE AGENT OF NON-A, NON-B HEPATITIS

In the absence of a specific serological test, evidence for the existence of more than one non-A, non-B agent rests on the demonstration of more than one acute episode of non-A, non-B hepatitis occurring in the same individual or, more indirectly, upon the observation of two or more distinct and reproducible clinical patterns among patients developing this disease. There has been some evidence to support each of these possibilities.

In 1977, Mosley et al evaluated the causes of 30 episodes of acute viral hepatitis occurring in 13 patients. No patient had more than one attack serologically defined as type B or type A hepatitis and none was due to CMV or EBV. Four patients appeared to have two distinct episodes of acute non-A, non-B hepatitis. Although it is possible that the second episode represented an acute recurrence of chronic non-A, non-B hepatitis, this did not seem to be the case. Each occurrence of non-A, non-B presented as a clinically distinct bout of acute hepatitis including the typical prodromal symptoms. More important, liver biopsies obtained during the second episode demonstrated acute rather than chronic hepatitis. These observations can be interpreted in at least three ways. First, there may indeed be at least two non-A, non-B agents. Second, recovery from non-A, non-B hepatitis may not be accompanied by the typical protective immunological response seen in most viral diseases, so that an individual might sustain repeated acute infections from the same agent. Third, some of the episodes may have represented non-viral hepatocellular injury; chemical injury would be the most likely since most of these patients were drug addicts. The hepatic histology in these cases was, however, typical of viral hepatitis rather than chemical injury, though these cannot always be reliably distinguished.

Shirachi et al (1978) suggested that there were two non-A, non-B agents based on their clinical presentation. Type-1 non-A, non-B infection had a short incubation period (mean 5.7 weeks) and a single (monophasic) rapid rise and fall of serum ALT, whereas type-2 infection was characterized by a

mean incubation period of 7.2 weeks and a biophasic, slowly resolving transaminase pattern. In addition, a new antigen was detected by agar gel diffusion in all 13 type-2 cases, but in only four of 10 type-1 patients. Whether or not this antigen actually represents a component of the agent responsible for non-A, non-B hepatitis has not been established (see below).

A provocative electron microscopic observation by Shimizu et al (1979) also suggests the possibility of at least two non-A, non-B agents. Plasma from a patient with acute non-A, non-B hepatitis (strain H) and from a patient with chronic non-A, non-B hepatitis (Strain F) were each inoculated into four chimpanzees. All eight chimpanzees developed evidence of non-A, non-B hepatitis. Thin-section EM of acute phase chimpanzee liver demonstrated intra-nuclear virus-like particles in four of four recipients of strain H and tubular structures with double unit membranes in the cytoplasm of four of four recipients of strain F. Each animal had one or the other of these morphological changes, but not both. This was also true of five additional animals who received other non-A, non-B inocula. In addition to these electron microscopic variations, strain H resulted in a disease with a shorter incubation period than that of strain F. While identification of the structures observed is at present uncertain, the morphological dichotomy suggests two distinct presentations for non-A, non-B hepatitis and, by inference, two distinct aetiological agents.

Many studies are now in progress to cross-challenge chimpanzees who developed non-A, non-B hepatitis with infectious material different from their original inoculum. If such animals develop a second histologically documented acute infection, this would provide additional evidence for more than one non-A, non-B agent. Conclusive demonstration of this occurrence cannot, however, be established until confirmed, specific serological markers for these agents are identified.

TREATMENT AND IMMUNOPROPHYLAXIS

There is no established treatment for non-A, non-B hepatitis. For the present, patients with acute non-A, non-B hepatitis should be managed according to the severity of their disease and with the same guiding principles applied to the management of acute type B hepatitis. There have been no studies which specifically investigate treatment modalities for non-A, non-B hepatitis. Although some patients have received steroids and/or cytotoxic agents, there is no evidence that these patients do better than those who are untreated. Drug therapy in such patients is extremely difficult to evaluate because most patients with chronic non-A, non-B hepatitis are only mildly symptomatic, because transaminase values fluctuate markedly as part of the natural history of this disease and because there is a tendency for spontaneous improvement. Considering the general benignity of the chronic course, the risk-benefit ratio of such drugs as steroids and cytotoxic immunosuppressants is marginal at best. Other agents such as interferon and adenine arabinoside, which are currrently being investigated for the treatment of chronic type B hepatitis, are not appropriate for investigational use in non-A, non-B hepatitis because of the lack of viral markers with which

to follow the effect ot therapeutic intervention. If, however, these agents prove successful in treating chronic type B hepatitis, they might be empirically applied to the treatment of severe cases of non-A, non-B hepatitis. For the present, and for the reasons cited above, I do not think prednisone or immunosuppressive agents should be utilized in the routine management of chronic non-A, non-B hepatitis. Clinically and histologically severe cases should be individually evaluated to determine if the risk-benefit ratio of drug therapy is favourable.

There have been a multitude of studies to evaluate the effectiveness of gamma globulin in the prevention of PTH. Results have been conflicting and this remains an area of controversy. Very few, if any, patients are currently receiving gamma globulin prophylaxis prior to transfusion. Only three studies have specifically looked at the effect of gamma globulin administration on the incidence and course of non-A, non-B PTH. A VA cooperative study (Seeff et al, 1977) suggested that immune serum globulin (ISG) significantly reduced the incidence of icteric non-B hepatitis, but did not significantly affect the total number of non-B hepatitis cases observed. Further, it could be shown in that study that the exclusion of commercial donors would have been a far more effective measure to prevent non-A, non-B hepatitis than would the administration of gamma globulin; indeed the concomitant use of a large number of commercial donors in this study confounds interpretation of the observed effect of ISG on icteric non-B hepatitis.

Knodell, Conrad and Ishak (1977) randomized patients to receive 10 ml of ISG or an albumin placebo prior to open heart surgery. They found both a decreased occurrence of icteric hepatitis and a lower incidence of total hepatitis (96 per cent non-A, non-B) in those receiving ISG as compared to placebo. In addition, only one of 22 acute non-A, non-B patients given ISG developed chronic liver disease as compared with nine of 22 who received the placebo ($P<0.01$). The results of this study have not been confirmed and, in fact, are somewhat contradicted by the study of Kuhns et al (1976), which showed no protective effect of ISG against non-B hepatitis in the transfusion setting. There were, however, differences in these studies in that Knodell et al administered 10 ml of ISG prior to transfusion whereas Kuhns et al gave 10 ml of ISG one week after transfusion and again four weeks after transfusion. Such discrepant data in the use of ISG for the prevention of PTH has plagued interpretation of these studies since their inception.

There have been no studies to evaluate ISG in the prevention of hepatitis after small volume exposure to blood from patients with non-A, non-B hepatitis such as might occur after accidental needlestick. The use of ISG in this situation is thus empirical, but is probably indicated in view of the extreme safety of this product.

PARTICLES, TESTS AND THE FUTURE

The identification of specific virus particles in both type B and type A hepatitis depended on their demonstration by immune electron microscopy. In the absence of specific immune aggregates, one cannot be certain if an

observed particle represents the specific virus being investigated. Immune electron microscopy in non-A, non-B hepatitis has been hampered by the absence of an antibody proven by other methods to be specific for the causative agent or agents. Investigators at the Hepatitis Laboratories, Center for Disease Control (1978), have, however, reported that convalescent phase serum from two patients with non-A, non-B hepatitis aggregated 27 nm particles in a liver homogenate prepared from a chimpanzee experimentally inoculated with factor VIII concentrate. These particles represent a candidate non-A, non-B virus. However, problems with reproducibility, questions regarding specificity of the immune aggregate and the possibility that other viruses were also transmitted in the inoculum which was derived from a pool of thousands of donors all dictate that this provocative finding be interpreted with caution.

There have been two published (Prince et al, 1978; Shirachi et al, 1978), and several unpublished reports of a serological test for the agent of non-A, non-B hepatitis. Each of these has, however, suffered from any or all of the following: lack of reproducibility, inability to be confirmed in other laboratories, inability to identify coded sera correctly, failure to demonstrate specificity for the infectious agent as opposed to specificity for liver-specific proteins or other serum or tissue proteins. It is the current consensus that there is no confirmed, valid serological test for the agent or agents of non-A, non-B hepatitis. Despite intensive efforts in this area and despite application of the same techniques which have been so successful in establishing antigenic markers for the type B virus, diagnostic serology for non-A, non-B has, for the most part, met with continued failure. There are several possible explanations for this. First, the amount of circulating viral antigen may be considerably less than that in type B hepatitis. Defective production of hepatitis B virions results in a massive excess of viral surface antigen as compared with the actual production of complete infectious (Dane) particles. This disproportionate production of HBsAg allowed for detection of this antigen by relatively insensitive methods such as agar gel diffusion. If defective viral production is not a feature of non-A, non-B hepatitis and the ratio of viral antigen to complete virion approaches unity, then the amount of circulating antigen will, in probability, be low and detection methods will have to be proportionately more sensitive. Second, non-A, non-B antigens may be complexed to immunoglobulin or other serum proteins and be undetectable for this reason. Third, the production of convalescent antibody against non-A, non-B antigens may be limited. As indicated in the section on chronic hepatitis and in Table 3, a very high proportion of non-A, non-B hepatitis patients have elevated serum transaminase for greater than one year after the onset of their disease and many have histological evidence of chronic hepatitis. It is probable that such patients do not develop antibody to the non-A, non-B agent. In addition to this large number of patients with biochemical and/or histological evidence of chronic liver disease, it is probable that there are other patients with normal transaminase values who are, nonetheless, chronic carriers of this virus and who also do not develop convalescent phase antibody. Indeed because of the above and because of fluctuating transaminase levels which

may demonstrate distinct elevations after long periods of normality, it is very difficult to ascertain which patients are truly convalescent and thus likely to have an antibody which could serve as a diagnostic reagent. We are now in a period of trial and error attempting to find the right combination of acute and convalescent phase sera which will react in established systems such as radioimmunoassay or enzyme-linked immunoassays. Alternative approaches will consist of identifying antigenic material in infected livers by immunofluorescence or of isolating antigen or antibody from circulating immune complexes.

As an interim measure, ALT determinations on donor blood may serve to identify some asymptomatic carriers capable of transmitting non-A, non-B hepatitis (Aach et al, 1978). However, in at least 50 per cent of cases of non-A, non-B PTH all donors have normal ALT values. In addition, approximately 60 per cent of patients receiving at least one unit of blood with an elevated transaminase do not develop hepatitis, either because they are not susceptible or because the donor transaminase elevation was of non-viral origin. A non-specific screening measure such as the ALT determination will thus fail to prevent at least 50 per cent of non-A, non-B cases and will result in a significant loss of donors who are not carriers of this presumed virus. Studies are currently underway to further evaluate the impact of donor ALT screening on the incidence of PTH and particularly to assess the consequences of such screening on the blood delivery system.

The establishment of a specific test for non-A, non-B is an item of highest priority and a primary goal for the immediate future. A test capable of detecting carriers of this agent or agents could, in combination with tests for HBsAg, markedly reduce, and indeed almost eliminate, the problem of post-transfusion hepatitis. Additionally, the discovery of a serological marker for non-A, non-B would allow progress in this area to parallel the incredible advances which had been made in relation to type B hepatitis. Not only would this help to better define the modes of non-A, non-B transmission, but would identify high-risk populations, would further elucidate the causes of chronic active hepatitis, cirrhosis and possibly hepatocellular carcinoma and might eventuate in the development of a non-A, non-B hyperimmune globulin and a non-A, non-B vaccine. These developments are obviously far off, but not beyond the realm of reason. An exciting new area in hepatitis research has surfaced and the potential for practical applications is enormous. Nonetheless, a major serological breakthrough is required before these exciting possibilities can be realized.

SUMMARY

Despite the exclusion of commercial donors and the introduction of sensitive radioimmunoassays for hepatitis B surface antigen, approximately 8 to 10 per cent of prospectively followed patients continue to develop post-transfusion hepatitis. Serological evaluation of these residual hepatitis cases showed them to be distinct from hepatitis B, hepatitis A, cytomegalovirus and the Epstein-Barr virus. These cases have been tentatively designated non-A, non-B and now compromise approximately 90 per cent of post-

transfusion hepatitis. There is increasing evidence that non-A, non-B hepatitis is also spread by routes other than blood. In the absence of a specific serological test, the diagnosis of non-A, non-B hepatitis depends on the clinical exclusion of non-viral causes of hepatocellular injury and on the serological exclusion of other known hepatitis viruses.

The clinical pattern of non-A, non-B hepatitis is characterized as follows: (1) The incubation period tends to be intermediate between type A and type B hepatitis, but considerable overlap exists. Most cases occur between five and 12 weeks after percutaneous exposure: (2) Non-A, non-B hepatitis tends to be less acutely severe than type B hepatitis; two thirds to three quarters of cases are anicteric, demonstrate only mild to moderate transaminase elevations and are symptomatically mild. Individual cases may, however, be clinically and biochemically severe and cannot be distinguished from acute type A or type B hepatitis. (3) A striking feature of non-A, non-B hepatitis is its propensity to progress to chronic liver disease. Up to 50 per cent of patients have elevated transaminase values in excess of one year and the majority of such patients, when biopsied, demonstrate histological features of chronic active hepatitis. Approximately 10 per cent show features of cirrhosis. Despite these histological changes, the chronic liver disease of non-A, non-B hepatitis seems, in most cases, to be slowly resolving rather than rapidly progressing.

Non-A, non-B hepatitis has now been repeatedly induced in chimpanzees using human acute and chronic phase inocula. These studies establish the existence of a transmissible agent and of a chronic asymptomatic carrier state. Transmission appears to be very similar to that for type B hepatitis. Additional studies in humans and chimpanzees suggest that there may be more than one non-A, non-B agent. At present, there is no confirmed serological test for detecting this agent(s). The development of such a test would represent a major breakthrough in the prevention of post-transfusion hepatitis and in better defining the epidemiological pattern and clinical consequences of this increasingly prevalent disease.

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