Transmission of Non-A, Non-B Hepatitis

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In studies conducted in the early 1950s, sera from six asymptomatic blood donors, implicated in the transmission of viral hepatitis, were inoculated into 10 to 20 volunteers each. Five of these "implicated" donor sera transmitted clinically apparent hepatitis to the recipients. The stored serum samples from these studies have been reanalyzed using serologic markers for hepatitis B virus and hepatitis A virus infection. Two of the donor sera were hepatitis B surface antigen (HBsAg)-positive, and both transmitted hepatitis B virus infection to all susceptible recipients, half of whom showed clinical symptoms. The remaining three infectious donors were HBsAg-negative, yet were icterogenic to 10% to 47% of recipients. Testing of serum samples from these recipients with hepatitis showed no evidence of hepatitis B virus or hepatitis A virus infection. This study and other recent evidence suggest that there is a third type of human viral hepatitis—non-A, non-B hepatitis—which is due to a transmissible agent and may well be associated with a chronic carrier state.

THE DISCOVERY and characterization of the hepatitis B surface antigen (HBsAg, Australia antigen) has allowed for clarification of much of the nature and epidemiology of type B hepatitis (1, 2). More recently, an antigen associated with viral hepatitis, type A, (HA Ag) has been identified and serologic methods developed for its detection (3, 4). Using these two serologic markers, it is now possible to positively identify an episode of hepatitis as having been either type A or type B hepatitis. Interestingly, such studies have shown that not all cases of viral hepatitis can be attributed to hepatitis A or hepatitis B virus (5-9). Furthermore, these non-A, non-B hepatitis cases cannot be accounted for by cytomegalovirus or Epstein-Barr virus infections. These findings suggest that there is at least a third human hepatitis virus transmitted by serum. However, the infectious nature of non-A, non-B hepatitis and its responsible agent have not as yet been shown.

The transmissibility of viral hepatitis by means of human serum was, of course, proved long before the discoveries of HBsAg and HA Ag. In studies conducted in the early 1950s, sera from several asymptomatic blood donors, who were suspected of having transmitted hepatitis to transfused recipients, were shown to be infectious when inoculated into human volunteers (10-12). The sera from these studies, which were collected 25 years ago, have been stored at −20 °C. The present study was undertaken to reanalyze those transmission studies using more-recently developed serologic techniques for the identification of type A and type B hepatitis.

Materials and Methods

Details of the original studies as well as clinical outcomes have been published previously (10, 11). These studies examined the possible infectivity of six asymptomatic blood donors, each of whom had been implicated in the transmission of viral hepatitis after a single-unit transfusion. The recipient of each unit of blood from these donors had developed clinically apparent hepatitis 32 to 70 days after transfusion, but none had received additional units of blood or plasma derivatives. The six "implicated" blood donors were contacted, questioned as to history of hepatitis or liver disease, examined for evidence of hepatic dysfunction, and had blood samples drawn for a battery of liver function tests. Blood samples obtained from these six donors at a point 43 to 385 days after the initial donation were stored frozen at −20 °C and used for transmission studies.

These transmission studies were carried out as a part of a large-scale study on means of inactivating hepatitis virus in blood (12). The recipients of the infectious materials were male inmates of federal penitentiaries who were between the ages of 21 and 40 and who had volunteered for these studies. Immediately before inoculation and weekly thereafter for at least 6 months, the recipients were questioned as to symptoms of hepatitis, examined for evidence of liver disease, and had blood drawn for various liver function tests (bilirubin, cephalin flocculation, and thymol turbidity)*. Residual serum specimens were stored frozen at −20 °C.

The present study is based upon the clinical records and liver-function-test data collected at the time of the original study as well as upon the results of serologic assays recently done on the stored serum samples from donors and recipients. Sera were tested for HBsAg by radioimmunoassay (13) and counterelectrophoresis (14). Subtyping of HBsAg was done on positive samples by immunodiffusion (15) and radioimmunoassay (16). The hepatitis B "e" antigen was assayed in selected sera by immunodiffusion (17). Antibody to HBsAg (anti-HBs) was tested by passive hemagglutination using human erythrocytes coated with HBsAg/adw (18). Samples giving equivocal results for anti-HBs were assayed by radioimmunoassay as well (19). Antibody to the hepatitis B core antigen (anti-HBc) was assayed in selected serum samples by complement fixa-

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* These studies were conducted from 1951 to 1954, before the development of the more sensitive assays of liver injury such as the serum transaminases, serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase.

tion (20). In those recipients developing hepatitis, a "pre" (first available sample) and "post" (sample taken 4 to 6 months after inoculation) sample were assayed for antibody to the hepatitis A antigen (anti-HA) by immune adherence hemagglutination using antigen purified from human stool (4, 21), for antibody to the Epstein-Barr virus (anti-EBV) by indirect immunofluorescence (VCA antigen: Bionetics Laboratories, Rockville, Maryland), and for antibody to the Ad-169 strain of cytomegalovirus (anti-CMV) by complement fixation.

For this study, the diagnosis of clinical hepatitis rested on the evaluations of the physicians who conducted the original study (10-12). Icteric hepatitis was diagnosed in any recipient developing a total serum bilirubin level of equal to or greater than 2.0 mg/dl. Hepatitis B virus infection was diagnosed by the development of HBsAg for 1 week or more or the appearance of anti-HBs or anti-HBc or a combination of these. The diagnosis of hepatitis A virus, cytomegalovirus, and Epstein-Barr virus infection rested on the appearance of antibody to these viral agents or a significant (four-fold) rise in antibody titers between preinoculation and convalescent serum specimens.

Results

DONORS

The six implicated donors (Table 1) were all men between the ages of 22 and 37. None gave a history of hepatitis or jaundice, although one (C.D.) reported mild fatiguability and had hepatomegaly on physical examination (10). Two of the donors (C.D. and V.S.) had a history of significant alcohol abuse, another (H.H.) was suspected of being a narcotic addict. Liver function tests showed normal bilirubin values in all, but five of the six had abnormal turbidity or flocculation tests or both. Sulfobromophthalein sodium (BSP) retention tests were done on four of the donors, all of which were abnormal. Three donors had liver biopsies, two of which showed mild periportal infiltrates and one of which showed minimal cirrhosis. Serum for HBsAg testing was available from five of the six donors. Two (H.H. and C.D.) were HBsAg-positive (both were subtype adw and were "e" antigen-positive). The remaining donors were HBsAg-negative and lacked anti-HBc as well. Serum was collected on these six implicated donors 43 to 385 days after the donation of blood that was implicated in the transmission of hepatitis.

RECIPIENT STUDIES

One millilitre of serum of each of the six donors was inoculated subcutaneously into ten to 20 volunteer recipients (Table 2). Serum from five of the six donors appeared to transmit hepatitis to the recipients, although the percentage of recipients developing clinical hepatitis varied with each donor inoculum (from 10% with V.S. and R.F. to 55% with H.H. serum) (11).

RECIPIENTS OF HBSAG-POSITIVE DONOR SERA

Serum from the HBsAg-positive donor, H.H., transmitted clinically-apparent hepatitis to 11 of the 20 recipients (55%) (Table 2). Serologic testing on the recipients, however, showed that all 20 developed hepatitis B virus infection or evidence of HBsAg exposure. Twelve of the 20 (60%) developed HBsAg (appearing 18 to 36 days after exposure and persisting 21 to 94 days). Ten of these 12 recipients developed clinical disease. In eight patients whose HBsAg could be subtyped, the HBsAg was of the subtype adw. Four recipients (20%) never developed HBsAg but showed a "primary antibody response" in that anti-HBs appeared for the first time 23 to 41 days after exposure, rose to high titer, and persisted (22). One of these four persons developed clinical symptoms. The remaining four had a "secondary antibody response," in that anti-HBs was present on the first bleeding and rose in titer with exposure (anamestic boost). None of these four patients developed clinical disease.

Serum from the second HBsAg-positive donor yielded similar results in these transmission studies. Three of 10 recipients (30%) of C.D. serum developed clinical disease (in three others anicteric hepatitis was suggested, but could not be documented at the time). However, serologic testing showed evidence of hepatitis B virus infection in all 10 recipients; eight (80%) developed HBsAg (25 to 81 days after exposure and persisting for 1 week to several years) and two (20%) developed a "primary antibody response" with anti-HBs appearing 25 to 39 days after exposure. HBsAg in four of these eight individuals could be subtyped, and in all four it was adw.

RECIPIENTS OF HBSAG-NEGATIVE DONOR SERA

Serum from the donor L.H. was HBsAg-negative, yet it induced clinically apparent hepatitis in seven of 15 recipients (47%) (Table 2). Testing of the sequential bleedings from these recipients did not show the development and persistence of HBsAg, anti-HBs, or anti-HBc. Simi-

Table 1. Data on Implicated Donors*

Donor	Age	Sex	History Jaundice	Abnormal Liver Function Tests				Liver Biopsy	Serum		Interval Between Donation and Serum Collection†
				TT	TF	CF	BSP		HBsAg	Anti-HBc	
	yrs						%				
H.H.	22	M	None	−	+	+	10.3	N.D.	+	+	135
C.D.	36	M	None	+	+	−	5.8	Cirrhosis	+	+	43
L.H.	25	M	None	−	−	−	5.4	Periportal infiltrates	−	−	385
V.S.	32	M	None	+	−	−	15.7	Periportal infiltrates	−	−	356
R.F.	37	M	None	+	+	−	N.D.	N.D.	−	−	149
W.C.	28	M	None	+	+	−	N.D.	N.D.	N.D.	N.D.	203

* TT = thymol turbidity, TF = thymol flocculation, CF = cephalin flocculation, BSP = sulfobromophthalein sodium retention at 45 min, N.D. = not done, HBsAg = hepatitis B surface antigen, anti-HBc = antibody to hepatitis B core antigen.
† Interval in days between date of "implicated" blood donation and date of serum collection for transmission studies.

Table 2. Data on Recipients of Implicated Donor Sera*

Donor Serum	Recipients	Recipients Developing Clinical Hepatitis			Recipients Developing			
		Icteric	Anicteric	Total	HBsAg	Primary Anti-HBs	Secondary Anti-HBs	Total
	no.		*no.*			*no.*		
H.H.	20	10	1	11 (55%)	12	4	4	20 (100%)
C.D.	10	2	1	3 (30%)	8	2	0	10 (100%)
L.H.	15	4	3	7 (47%)	0	0	0	0
V.S.	10	1	0	1 (10%)	0	0	0	0
R.F.	10	1	0	1 (10%)	0	0	0	0
W.C.	10	0	0	0	0	0	0	0

* Primary anti-HBs, development of antibody to hepatitis B surface antigen (anti-HBs) for the first time 3 to 12 weeks after exposure; secondary anti-HBs, presence of anti-HBs on preinoculation bleedings and rise in titer (anamnestic boost) with exposure. HBsAg = hepatitis B surface antigen.

larly, donors V.S. and R.F. were both HBsAg-negative yet both transmitted icteric hepatitis to one of 10 recipients. In none of these patients, however, did serologic evidence of hepatitis B virus infection occur. Finally, among 10 recipients of serum from donor W.C., none developed clinical hepatitis or serologic evidence of type B hepatitis.

The nine individuals who developed clinical hepatitis that could not be attributed to hepatitis B virus were studied for evidence of infection with hepatitis A virus, cytomegalovirus, and the Epstein-Barr virus. These data along with specific results of HBsAg testing are shown in Table 3. Serologic testing of early and late bleedings from these nine individuals showed no evidence of infection with either hepatitis B virus or hepatitis A virus. Among these nine recipients, two had anti-HBs, three anti-HBc, and eight anti-HA on preinoculation bleedings. Titers of these antibodies did not rise subsequent to clinical hepatitis, and those individuals without pre-existing antibody did not seroconvert.

CLINICAL FEATURES IN RECIPIENTS

The clinical disease seen in these nine persons with "non-A, non-B" hepatitis was in most respects similar to viral hepatitis, type B. The typical symptoms of anorexia, malaise, nausea, vomiting, and abdominal pain were seen with equal frequency in both types of hepatitis (Table 4). Fever was notably absent; headache and myalgias were rare. Hepatomegaly was noted in most instances. In general, however, the non-A, non-B hepatitis cases were milder than type B cases (Figures 1 and 2). The duration of symptoms and jaundice, as well as peak bilirubin levels, were on the average lower in the non-A, non-B cases. Three of the nine (33%) non-A, non-B cases were anicteric, as opposed to two of 14 (14%) type B hepatitis cases. Among the 14 cases of type B hepatitis, there were two that were judged at the time to be "severe" (both developed coma); among the nine non-B cases, none was rated as more than mild to moderate in clinical severity.

The incubation periods in each recipient developing hepatitis in response to each of the five implicated donor sera are shown in Figure 3. Incubation periods for type B hepatitis cases ranged from 35 to 77 days, and for non-A, non-B cases 18 to 89 days. The incubation periods (from date of exposure to onset of clinical symptoms) in those individuals receiving the L.H. serum (averaging 40 days) were on the whole shorter than those seen in recipients of either H.H. or C.D. serum (averaging 52 to 62 days), although these differences were not statistically significant.

REINOCULATION STUDIES

In order to evaluate immunity and the possibility of reinfection, selected individuals who had developed hepatitis after an initial inoculation were reinoculated with the same implicated donor serum 10 months later (Table 5). Five persons initially given serum from the implicated donor H.H. (HBsAg-positive) and five others initially given serum from chronic carrier donor L.H. (HBsAg-negative) were rechallenged with the same infectious serum. Those individuals receiving a second inoculation with H.H. serum all had anti-HBs at the time of rechal-

Table 3. Serologic Data on Nine Recipients with Non-A, Non-B Hepatitis*

Donor Inoculum	Recipient No.	HBsAg	Anti-HBs		Anti-HBc		Anti-HA		Anti-CMV		Anti-EBV	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
L.H.	M 12	—	1:128	1:256	1:64	1:32	≥1:1000	≥1:1000	0	0	1:10	1:10
	M 13	—	0	0	0	0	0	0	0	0	0	0
	M 14	—	0	0	0	0	1:10	1:10	0	0	1:160	1:160
	M 17	—	1:16	1:16	1:8	1:16	1:640	1:800	1:32	1:16	1:10	1:10
	M 19	—	0	0	1:8	1:16	1:800	1:800	1:8	1:16	1:40	1:40
	M 20	—	0	0	1:16†	1:8†	≥1:1000	1:100	1:32	1:16	1:40	1:40
	L 175	—	0	0	0	0	1:100†	1:10†	0	0	1:40	1:40
V.S.	M 42	—	0	0	0	1:8†	≥1:1000	≥1:1000	1:16	1:16	1:640	1:640
R.F.	M 5	—	0	0	0	0	1:1000	1:100	0	0	1:10	1:10

* HBsAg = hepatitis B surface antigen, anti-HBs = antibody to HBsAg, anti-HBc = antibody to hepatitis B core antigen, anti-HA = antibody to hepatitis A antigen, anti-CMV = antibody to cytomegalovirus, anti-EBV = antibody to Epstein Barr virus.
† Sample agglutinates control cells at an identical titer.

Table 4. Comparison of Clinical Features of Hepatitis Cases: Type B versus Non-A, Non-B

Hepatitis Type	Donor Inocula	Cases of Hepatitis	Patients with Hepatitis who Developed:					
			Anorexia	Malaise	Nausea	Vomiting	Abdominal Pain	Hepatomegaly
		no.	←			*%*		→
Type B	H.H., C.D.	14	93%	93%	100%	78%	100%	86%
Non-A, non-B	L.H., V.S., R.F.	9	89%	67%	78%	67%	89%	78%

lenge, and each showed a boost in titer of anti-HBs (titers of anti-HBc, however, did not change). One recipient redeveloped HBsAg for a 2-week period (10 to 12 weeks after reinoculation), but did not have a recurrence of clinical symptoms, and bilirubin levels and flocculation-turbidity tests did not change.

Rechallenge of five individuals with serum from the donor L.H. (HBsAg-negative) was not associated with the development of clinical disease, HBsAg, anti-HBs, nor anti-HBc. Two of these recipients had anti-HBs and anti-HBc before rechallenge (as well as before initial inoculation); titers of these hepatitis B virus-related antibodies did not change with rechallenge with the HBsAg-negative L.H. serum.

EVIDENCE OF PERSISTENT LIVER DISEASE

Follow-up examinations, including history, physical examination, blood tests, and BSP retention, done 1 to 3 years after the initial inoculation, were available from 10 of the 14 recipients with type B hepatitis and from six of the nine recipients with non-A, non-B hepatitis. Evidence of chronic liver disease in the form of mild symptoms of fatiguability, hepatomegaly, and abnormal turbidity/flocculation tests was found in one of the 10 recipients (10%) who had developed type B hepatitis and in two of six (33%) recipients who had developed non-A, non-B hepatitis. However, none of these individuals had an elevated bilirubin level (none was > 1.0 mg/dl) or BSP retention (all were $\leq 5\%$ at 45 min).

Discussion

Analyses of cases of type B hepatitis in this study add little to what is known about this disease. Of interest is that the two HBsAg-positive sera were infectious to 100% of recipients. The fact that only 47% (14/30) of the recipients developed jaundice or clinical symptoms reflects the frequently asymptomatic nature of type B hepatitis. Indeed the most striking feature of these type B hepatitis cases was the variability of clinical and serologic outcomes. Despite inoculation with the same infectious serum, a great range of clinical disease was seen: from a simple development of anti-HBs without symptoms and without detectable HBsAg in five recipients, to the development of the chronic carrier state in one individual, to the development of fulminant hepatitis in two. This suggests that the determinants of severity and clinical outcome in type B hepatitis are factors of the host rather than factors of the virus (virus strain, "virulence," dose, or route of exposure). In this study, as in others, the subtype of HBsAg "bred true," that is, the recipients developed the same subtype as that found in the inoculum (15, 16). In a reinoculation study, immunity in type B hepatitis was shown with one interesting exception. One patient convalescent from an anicteric episode of type B hepatitis redeveloped HBsAg after reexposure. This second period of antigenemia was brief and not associated with clinical disease. Thus, immunity to reinfection with hepatitis B virus did not appear to be absolute, but reinfection was mild and asymptomatic.

The present study adds support to the growing evidence for a third type of human viral hepatitis (5-9). Sera from five blood donors that had been implicated in the transmission of post-transfusion hepatitis were shown to transmit clinically apparent, viral hepatitis to recipients. Two of these five donors were HBsAg-positive, clearly chronic

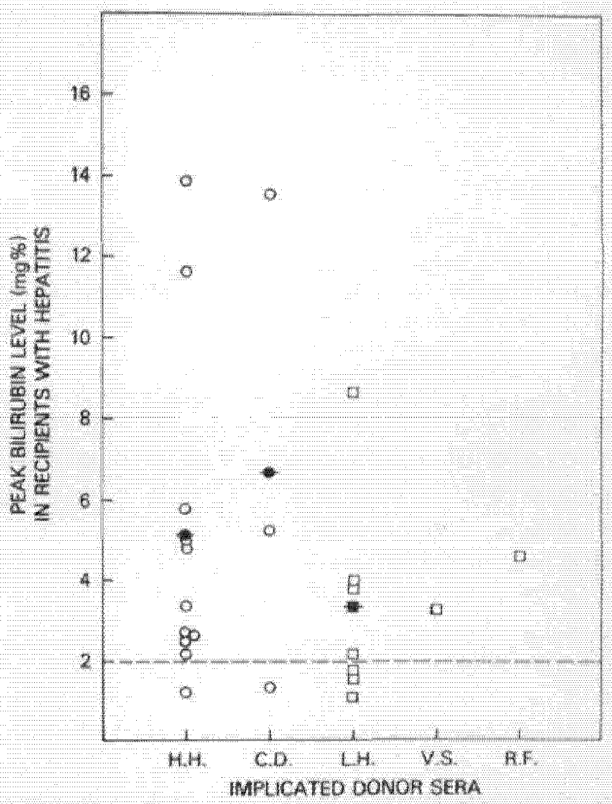

Figure 1. Peak bilirubin level in mg% (mg/dl) for each case of clinically apparent hepatitis in the recipients of each of the five implicated donor sera. Circles indicate type B hepatitis cases; squares indicate non-A, non-B hepatitis cases; closed, scored symbols indicate the mean of each group.

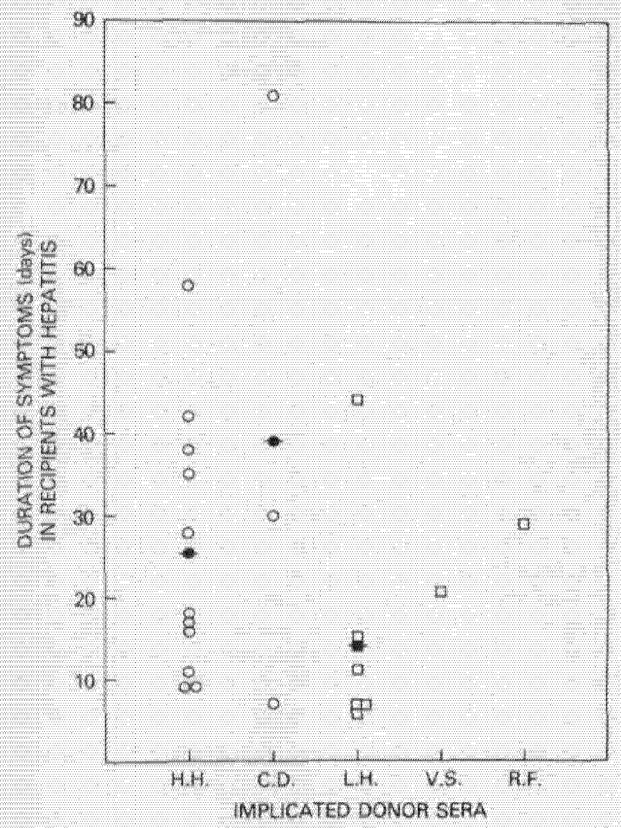

Figure 2. Duration of clinical symptoms in days for each case of clinically apparent hepatitis in the recipients of each of the five implicated donor sera. Circles indicate type B hepatitis cases; squares indicate non-A, non-B hepatitis cases; closed, scored symbols indicate the mean of each group.

HBsAg carriers, and their sera transmitted type B hepatitis with regularity. The remaining three infectious donors were HBsAg-negative, but their sera were, nonetheless, icterogenic transmitting a form of hepatitis due neither to hepatitis A virus nor hepatitis B virus as judged by sensitive serologic tests on serial samples from recipients. One of these infectious donor sera (L.H.) transmitted hepatitis in seven of 15 recipients, whereas the other two transmitted this disease less frequently (in one of 10 recipients each). The clinical disease seen in the recipients of the HBsAg-negative sera was similar to viral hepatitis, type B, but HBsAg, anti-HBs, and anti-HBc were not found in association with this disease.

Serologic assays on the sera from these patients with "non-B" hepatitis failed to show evidence of hepatitis A virus infection. Eight of the nine individuals who developed "non-B" hepatitis possessed antibody to hepatitis A antigen on preinoculation bleedings. Titers of anti-HA did not change with the episode of clinical hepatitis, and the single recipient without pre-existing anti-HA did not seroconvert. This is strong serologic evidence that this "non-B" hepatitis was also "non-A". It has been argued, however, that there may be different strains or serotypes of hepatitis A virus, and that the serologic tests developed for the detection of anti-HA may reflect antibody to one strain only (23). This possibility is difficult to disprove, yet it is unlikely in light of the accumulating data generated using the presently available assays for hepatitis A antigen and antibody. Analyses of outbreaks of typical type A hepatitis (from contaminated food, drinking water, shellfish, and from experimental transmission studies in man and primates) have shown the regular development of antibody to hepatitis A antigen derived from either liver or stool (3, 4, 8, 24-28). Furthermore, epidemiologic and clinical features of non-A, non-B hepatitis suggest that it is a disease distinct from type A hepatitis. Non-A, non-B hepatitis occurs in settings that are not typical of type A hepatitis; most cases have been described in association with parenteral exposure. Unlike type A hepatitis, published cases of non-A, non-B hepatitis have not been associated with secondary spread of the disease to contacts (5, 7). Immune serum globulin ("gamma globulin"), which is clearly effective in preventing type A hepatitis, has been shown to have little or no effect in preventing non-A, non-B hepatitis after blood transfusions (29-31). The incubation period of most cases of non-A, non-B hepatitis (averaging 6 to 9 weeks) has been longer than that generally accepted for type A hepatitis (averaging 2 to 6 weeks) (5-7, 32). Finally, the symptoms of fever, headaches, and myalgias, which are so common with type A hepatitis (32, 33), appear to be rare

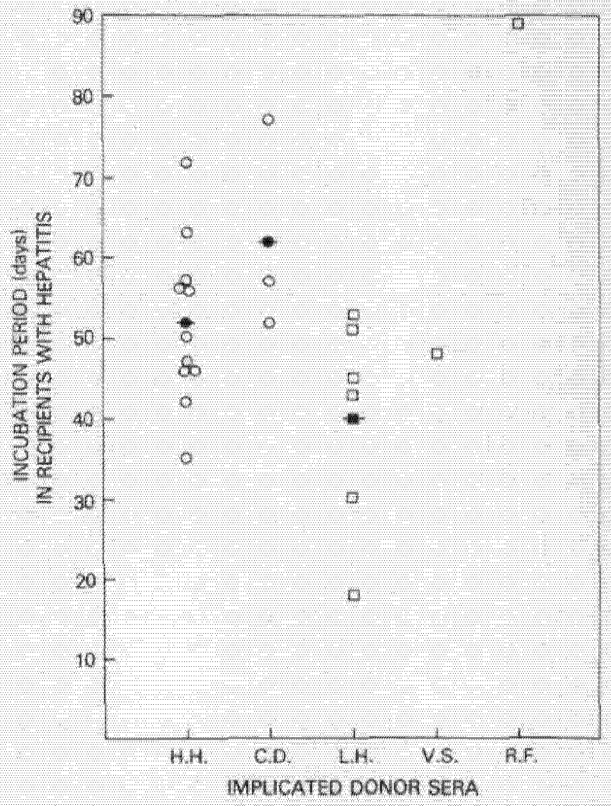

Figure 3. Incubation period in days (from day of exposure to day of symptom onset) for each case of clinically apparent hepatitis in recipients of each of the five implicated donor sera. Circles indicate type B hepatitis cases; squares indicate non-A, non-B hepatitis cases; closed, scored symbols indicate the mean of each group.

Table 5. Reinoculation Studies

Hepatitis Type	Donor Serum	Recipients Reinoculated	Recipients Developing Clinical Hepatitis		Recipients Developing*		
			Icteric	Anicteric	HBsAg	Primary Anti-HBs	Secondary Anti-HBs
		←		no.			→
Type B	H.H.	5	0	0	1	0	4
Non A, non B	L.H.	5	0	0	0	0	0

* HBsAg = hepatitis B surface antigen, anti-HBs = antibody to HBsAg.

in non-A, non-B hepatitis. It is thus unlikely that these cases represent a variant of type A hepatitis.

The cytomegalovirus and Epstein-Barr virus can both induce a hepatitis like syndrome (34, 35), but the liver function abnormalities due to these viruses are usually mild and overshadowed by other symptoms and signs (pharyngitis, adenopathy, splenomegaly). In this study, symptoms and signs of these viral infections were absent, and antibody responses to these two viruses were not seen. Furthermore, the icterogenic material used in this study was serum; transmission of cytomegalovirus and Epstein- Barr virus infection by transfusion has been attributed to the transfer of virus-bearing leukocytes (34, 35).

Attempts to identify a viral antigen or particle associated with non-A, non-B hepatitis have so far been unsuccessful. Because it is not known whether one or many (or no) viral agent is responsible for this disease and because it is defined by the *lack* of HBsAg and the nonappearance of anti-HA, the term "non-A, non-B hepatitis" has gained wider use than "type C hepatitis" or "viral hepatitis, type C" (5-9). Indeed, it has not been proved that non-A, non-B hepatitis is due to a transmissible agent. Most reports on this disease have been from studies on patients receiving multiple unit transfusions (5-7), hemophiliacs (36), and intravenous-drug addicts (9). These are groups that are prone to develop other, nonviral types of liver injury. The possibilities of coincidental drug-related liver disease, alcoholism, iron-overload, passive congestion of the liver, "immunologic" liver disease, or exacerbation of an underlying, idiopathic chronic hepatitis have to be considered as explanations of this syndrome of acute liver disease.

More and more evidence, however, indicates that non-A, non-B hepatitis is due to a transmissible agent that is most likely a virus. Non-A, non-B hepatitis is regularly associated with transfusion, accounting for at least half of post-transfusion hepatitis cases (6, 7, 30, 31, 37, 38). Non-A, non-B hepatitis is clinically similar to type B hepatitis and has a similar incubation period (5-9). Finally, non-A, non-B hepatitis has been shown to occur more frequently after transfusion of commercial donor blood than voluntary donor blood (7, 30, 37). The present study adds support to the data suggesting that non-A, non-B hepatitis is due to a transmissible agent. One-millilitre amounts of three "implicated" donor sera transmitted clinically recognizable hepatitis to human volunteers. An incubation period suggestive of typical viral hepatitis was seen in each case. The clinical disease was in most regards similar to type B hepatitis, but HBsAg, anti-HBs, and anti-HBc did not appear. Reinoculation of five of these patients with the original icterogenic serum did not induce a recurrence of the disease, suggesting the acquisition of immunity from the original episode of hepatitis. The reproducibility of this viral hepatitislike syndrome in these healthy volunteers argues strongly for the presence of a transmissible agent and against a coincidental drug-related, toxic, or chronic hepatitis.

Several clinical and epidemiologic features of non-A, non-B hepatitis have become clear from studies such as the present one. First, non-A, non-B hepatitis closely resembles type B hepatitis. The incubation period, the clinical symptoms and signs, and the potential for chronicity appear to be similar to type B hepatitis (5-9). Undoubtedly, what was once referred to as "serum hepatitis" included both type B and non-A, non-B hepatitis. Second, non-A, non-B hepatitis appears to be spread predominantly by the parenteral route. Most cases have been described in association with transfusion, intravenous drug use, or serum inoculation (5-7, 30, 31, 35-37). However, as in type B hepatitis, the importance of "nonparenteral" routes of transmission (by saliva, sexual and intimate contact, biting insects) needs to be assessed (8, 9). Third, non-A, non-B hepatitis appears to be associated with a chronic carrier state and chronic liver disease. In this study, sera taken from HBsAg-negative donors 149 to 385 days after an implicated transfusion were found to be infectious. These "implicated" blood donors were, for the most part, asymptomatic, although liver function tests and liver biopsy examinations frequently showed evidence of underlying chronic hepatitis. Finally, non-A, non-B hepatitis appears to be common. Three of the five infectious donors studied here transmitted this non-A, non-B hepatitis. Previous studies on post-transfusion hepatitis have shown that 40% to 71% of such hepatitis is non-A, non-B (6, 7, 30, 31, 37, 38). Currently, all blood donations are screened for HBsAg by radioimmunoassay (or a method of similar sensitivity). Data generated from post-transfusion hepatitis studies done since the institution of such sensitive screening methods suggest that at the present time more than 90% of post-transfusion hepatitis is due to non-A, non-B hepatitis (6, 30). All these features suggest the presence of one or more other human hepatitis viruses and insure a continued interest and activity in the field of viral hepatitis.

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