NON-A, NON-B VIRAL HEPATITIS

Paul V. Holland, M.D., and Harvey J. Alter, M.D.†*

Non-A, non-B hepatitis is a newly recognized disease entity. Although initially described as a transfusion related viral infection, the disease can occur in sporadic, endemic, and epidemic settings. There are no confirmed, reproducible serologic tests for associated antigens or antibodies, but electron microscopy has revealed virus-like particles of different sizes. Nonspecific laboratory tests of hepatic dysfunction, especially alanine aminotransferase, are currently utilized to diagnose non-A, non-B hepatitis in patients and may be used to implicate blood donor carriers of this virus. The existence of an infectious non-A, non-B hepatitis agent and proof of a chronic carrier state in humans have been documented by transmission studies in chimpanzees. Cross challenge studies in chimpanzees, as well as some epidemiologic data, suggest that more than one agent causes non-A, non-B hepatitis. Hum Pathol 12:1114–1122, 1981.

For years viral hepatitis was considered to be caused primarily by one of two hepatotropic viruses, either virus A (infectious hepatitis) or virus B (serum hepatitis).[1] On occasion infections with the Epstein-Barr virus (infectious mononucleosis) and the cytomegalovirus also result in hepatic inflammation.[2,3] Now, however, studies of transfusion associated viral hepatitis and the identification of antigens and antibodies specifically related to viral hepatitis types A and B have made possible the recognition of a third major cause of human viral hepatitis.[4–7] This entity, diagnosed by serologic exclusion, has been tentatively designated non-A, non-B hepatitis.

In the absence of reproducible serologic markers for evidence of non-A, non-B infections, the term "non-A, non-B hepatitis" has been used in preference to a more definitive designation, e.g., hepatitis C. The term non-A, non-B hepatitis does not limit the etiology of this disease to a single agent; in fact, there is already evidence of the existence of more than one type of non-A, non-B hepatitis.[8] As specific tests become available for identifying infection with, or immunity to, non-A, non-B hepatitis viruses, more definite terms can be applied to each etiologic agent.

This review attempts to summarize the current state of our understanding of non-A, non-B hepatitis. It covers primarily the clinical and epidemiologic aspects of this disease, but also summarizes the status of recently reported tests for non-A, non-B hepatitis associated antigens and antibodies. Finally, it analyzes available means for decreasing the occurrence of this disease as a transfusion transmitted viral illness.

EPIDEMIOLOGY OF NON-A, NON-B HEPATITIS

Transfusion of Blood Products

Transfusion transmitted viral hepatitis has been presumed to be due primarily to the hepatitis B virus.[1] However, the incubation periods in transfusion hepatitis cases suggested that more than one virus might be involved.[9] The expected unimodal curve for incubation periods was not observed; instead a broad, skewed curve more likely due to multiple virus agents was noted. Without specific virologic markers, the possibility of multiple etiologic agents of transfusion hepatitis remained speculative.

The discovery of the Australia antigen and its subsequent association with viral hepatitis type B revealed that a minority of the cases of hepatitis related to transfusions was caused by the hepatitis B virus.[7,10] This was true even before widespread screening of blood donors for what came to be called the hepatitis B surface antigen (HBsAg). The application of sensitive assays for serologic markers related to hepatitis B virus–HBsAg, as well as antibody to HBsAg (anti-HBs) and antibody to the hepatitis B core antigen (anti-HBc), showed that only 25 to 30 per cent of the patients with transfusion associated hepatitis had infections due to this agent.[11] With the advent of HBsAg screening of all blood donors and rejection of those positive for this antigen, the incidence of type B hepatitis fell still further.[12] Hepatitis B virus now accounts for less than 10 per cent of the cases of transfusion related hepatitis.[13]

Accepted for publication July 20, 1981.

* Clinical Associate Professor of Medicine, George Washington University School of Medicine and Health Sciences, Clinical Associate Professor of Pathology, Georgetown University School of Medicine, Washington, D.C. Director, Blood Bank Department, Clinical Center, National Institutes of Health, Bethesda, Maryland.

† Clinical Associate Professor of Medicine, Georgetown University School of Medicine, Washington, D.C. Chief, Immunology Section, Blood Bank Department, Clinical Center, National Institutes of Health, Bethesda, Maryland.

0046-8177/81/1200-1114 $01.80 © W. B. Saunders Co.

Type A viral hepatitis is usually transmitted by the fecal-oral route in epidemic situations.[1] It is unlikely to be transmitted by a blood transfusion, because a chronic asymptomatic carrier state for hepatitis A virus has not been documented. Nonetheless the discovery of an antigen and antibody related to hepatitis A virus infections permitted a more detailed evaluation of non-B viral hepatitis caused by transfusions and revealed that virtually no cases were due to hepatitis A virus.[5,6] Only on rare occasions has hepatitis A virus been transmitted by transfusion, presumably by blood donors during the incubation period of hepatitis A virus infection.[14]

Modes of Spread of Non-A, Non-B Hepatitis

Blood transfusion was the first recognized mode of spread of non-A, non-B hepatitis and remains a major means of viral dissemination at the present time. However, because most carriers of non-A, non-B hepatitis have not themselves been transfused, other means must exist for the acquisition of this disease. The lack of hepatitis A and B viral markers in many patients with viral hepatitis unrelated to blood transfusion also suggests that non-A, non-B hepatitis can be acquired without needlestick exposure.

Non-A, non-B hepatitis can occur in sporadic, endemic, and even epidemic situations. From 13 to 25 per cent of the cases of sporadic hepatitis that are sufficiently severe to cause patients to seek medical attention are due to non-A, non-B hepatitis virus.[15,16] The mode of spread is frequently unknown in such patients, although some may be due to sharing of contaminated needles by intravenous drug users.[15,16] The epidemiology of non-A, non-B hepatitis does not suggest fecal-oral transmission. For example, there is infrequent spread of non-A, non-B hepatitis to family or other close contacts,[17,18] and except for some rare instances, to be noted, there are no common source epidemics.[19,20] Accidental needlesticks with minute quantities of blood have resulted in transmission of non-A, non-B hepatitis to health care workers,[21,22] but common exposure to fomites, e.g., CPR mannikins used by individuals incubating non-A, non-B hepatitis, has not.[23] The minimal intrafamilial spread of non-A, non-B hepatitis that has been observed may be due to inapparent parenteral transmission, e.g., shared razors or toothbrushes, or to sexual transmission, since spouses appear to be at somewhat higher risk.[18] Overall, the transmission of non-A, non-B hepatitis appears similar to that of the type B hepatitis virus and generally dissimilar to that of hepatitis A virus, at least in the United States. However, in the absence of serologic markers, precise transmission patterns cannot be defined.

Heretofore, all epidemics of viral hepatitis have been presumed to be due solely to hepatitis A. When serologic studies were performed, antibody responses to hepatitis A virus have been detected in epidemics of viral hepatitis.[24] Recent studies from India, however, implicate non-A, non-B hepatitis as a cause of some epidemics of viral hepatitis.[19,20] In three presumably water borne, common source epidemics of viral hepatitis, there was no evidence of hepatitis A and little evidence of hepatitis B infection. The clinical picture of acute viral hepatitis was typical for type A disease except that a few patients died with fulminant hepatic failure.[20] When liver biopsies were performed in individuals with acute epidemic non-A, non-B hepatitis, an unusual pattern was noted; many had cholestatic features with "glandlike" transformation of hepatocytes.[20] Another unusual feature of epidemic non-A, non-B hepatitis is that chronic liver disease was virtually absent as a sequela.[25] This is in marked contrast to the high frequency of chronicity noted after transfusion acquired non-A, non-B hepatitis (see p. 1118).[26] Henceforth, epidemics of hepatitis will have to be evaluated for evidence of hepatitis A virus exposure before being presumed to be due to this virus.

DIAGNOSIS OF NON-A, NON-B HEPATITIS

Most of our information about the clinical aspects of non-A, non-B hepatitis has been derived from studies of transfusion transmitted disease. With transfusions, the precise time of exposure to non-A, non-B disease could be readily identified; in addition, many transfused patients were followed prospectively with frequent laboratory examinations to detect evidence of hepatic dysfunction. In the transfusion setting it has been shown that the incubation period of non-A, non-B disease can be as short as two weeks and as long as 26; however, the majority of cases occur between five and 10 weeks after transfusion.[26] The incubation period of non-A, non-B hepatitis is, on average, longer than that for hepatitis A virus infection and somewhat shorter than that for hepatitis B virus disease. The onset of disease is typically insidious and most often anicteric. Less than 25 per cent of the patients have overt disease with jaundice.[26] If present, symptoms are usually nonspecific, anorexia and easy fatigability being the most prominent complaints.

Reproducible serologic tests to diagnose non-A, non-B hepatitis are not available. Nonspecific tests of hepatic injury, particularly alanine aminotransferase (ALT, SGPT), are employed to document the hepatic inflammation of non-A, non-B hepatitis. In the presence of an elevated alanine aminotransferase level and in the absence of congestive failure, anoxia, hepatotoxic medication, alcoholism, or evidence of hepatitis B virus infection, non-A, non-B hepatitis should be highly suspect as the cause of the elevated alanine aminotransferase level. The alanine aminotransferase elevation need not be marked, although individual patients may have peak values 20 or more times the upper limit of normal. On the average, the mean peak alanine aminotransferase level associated with non-A, non-B hepatitis is less than that in hepatitis B virus infection, but the overlap of alanine

aminotransferase levels between the two diseases is considerable.[26] A frequent characteristic of non-A, non-B hepatitis is the tendency for the serum alanine aminotransferase level to fluctuate dramatically.[27] Wide fluctuations from normal to markedly abnormal may be seen over relatively brief time intervals. In those who go on to develop chronic hepatitis there is a tendency for the magnitude of fluctuation to gradually diminish over a period of months to years.[28]

The mean duration of transfusion acquired non-A, non-B disease in patients who recover from the disease is 10 weeks.[26] This duration of non-A, non-B disease is slightly longer than that of transfusion transmitted type B disease (eight weeks). By contrast, in sporadic cases of viral hepatitis requiring hospitalization, patients with non-A, non-B hepatitis recover sooner than those with type B disease.[15] When non-A, non-B hepatitis occurs in an epidemic situation, recovery occurs in two to six weeks.[20]

The most striking clinical feature of non-A, non-B hepatitis is its predilection for becoming chronic. This is true of non-A, non-B hepatitis acquired in the transfusion setting and to a lesser extent when non-A, non-B hepatitis is acquired sporadically.[15,26] Whereas virtually all patients recover from type A hepatitis, and not more than 10 per cent have chronic disease after type B hepatitis, 25 to 50 per cent of the patients with transfusion associated non-A, non-B hepatitis have elevated alanine aminotransferase levels for more than one year after the onset of the disease.[29] This striking propensity for non-A, non-B hepatitis acquired by transfusion to become chronic has not been noted in the few epidemics attributed to this virus (or viruses).[25] Patients with chronic non-A, non-B hepatitis are generally asymptomatic and are unaware of their alanine aminotransferase elevations, but liver biopsies have further documented the existence of chronic hepatitis.[29] The most prevalent finding on liver biopsy is the pattern of chronic active hepatitis. In about one-third of the patients, chronic persistent hepatitis or nonspecific hepatic changes have been noted. Patients with chronic active hepatitis may progress to frank cirrhosis; in the majority, however, there is apparently a very slow resolution to a more benign histologic lesion.

Individuals with persistently elevated alanine aminotransferase levels after non-A, non-B hepatitis appear to be chronic carriers of the virus, as documented by chimpanzee transmission studies.[22,30] However, the alanine aminotransferase level in non-A, non-B hepatitis virus carriers need not remain above the upper limit of normal. A well documented carrier state has been present for at least six years in one individual; his serum continued to transmit non-A, non-B hepatitis to chimpanzees even after the alanine aminotransferase level returned to the normal range.[22] The frequent occurrence of chronic hepatitis or a carrier state after non-A, non-B hepatitis has undoubtedly complicated efforts to identify convalescent antibody for serologic tests to a non-A, non-B virus.

Differential Diagnosis of Non-A, Non-B Hepatitis

Since nonspecific laboratory tests of hepatic dysfunction must be used in suspected cases of non-A, non-B hepatitis, other causes of abnormal tests must be ruled out. Serologic tests for known etiologic agents of viral hepatitis must be performed to exclude hepatitis A virus, hepatitis B virus, Epstein-Barr virus, and cytomegalovirus infections. Toxic, drug, nonviral, and allergic causes of hepatitis must be considered and ruled out. Congestive heart failure as well as other nonspecific causes of elevated transaminase levels must also be excluded. With an appropriate clinical setting, e.g., transfusions or needlestick exposure, non-A, non-B hepatitis can be presumed when transaminase levels are elevated, when hepatitis A and B viral markers are absent, and when there is no other evident nonviral cause of the elevations.

It is unusual for a patient with acute viral hepatitis of any etiology to undergo liver biopsy. Thus, the pathologic features of acute non-A, non-B hepatitis are not well characterized. Usually it is only when the disease persists longer than six months that a liver biopsy is performed.[29] The pathologic examinations then reveal a spectrum of findings consistent with resolving hepatitis, chronic persistent hepatitis, and chronic active hepatitis with or without early cirrhosis.[27,29,31]

During an epidemic of non-A, non-B hepatitis in India 28 patients with acute viral hepatitis underwent liver biopsy.[20] Khuroo[20] noted that in 14 biopsies there were features of "classic viral hepatitis," in another four "severe viral hepatitis with bridging," and in 10 a "cholestatic form of viral hepatitis with intracanalicular bile stasis and rosette formation of hepatocytes as the dominant features." The latter biopsy specimens had "glandlike transformation of hepatocytes," which were thought to be distinctive and identical to findings in liver biopsy specimens from patients with acute viral hepatitis acquired during the Delhi epidemic of 1955–1956.[32] Serum specimens taken from patients during this Delhi epidemic were subsequently examined for hepatitis A and B virus markers; it appears this huge epidemic was due to non-A, non-B hepatitis.[19]

SPECIFIC MEANS FOR DETECTING NON-A, NON-B HEPATITIS VIRUS

Infection of chimpanzees documented a transmissible agent in non-A, non-B hepatitis.[30] This agent is presumed to be a virus because of the lack of evidence of bacterial or fungal infection, the demonstration that the agent can pass through a 0.22 μ filter, and the histologic features, which are very similar to those in type A and type B viral hepatitis.[33]

Transmission of non-A, non-B hepatitis to chimpanzees, although not a readily available method, is at present the only reproducible means of demonstrat-

TABLE 1. SIZE OF PUTATIVE NON-A, NON-B HEPATITIS VIRUS PARTICLES

Author	Material	Size	Comments
Prince (1978)*	Liver	60–80 nm.	
Tabor (1978)*	Liver	20–22 nm.	Cytoplasmic?
Bradley et al. (1979)	Serum, liver, and factor VIII	25–30 nm.	Some full, some empty
Shimizu et al. (1979)	Liver	20–27 nm.	Intranuclear
Mori et al. (1980)	Serum	32 nm. with 22 nm. inner core	Free core particles
Hantz et al. (1980)	Serum	15–25 nm. and 35–40 nm.	20–24 nm. Larger particles have a double shell and dense inner core
Yoshizawa et al. (1980)	Fibrinogen and serum	25–29 nm.	

* Described during non-A, non-B hepatitis workshop in *Viral Hepatitis*, edited by Vyas, Cohen, and Schmid.

ing that an inoculum is infectious for a non-A, non-B hepatic agent. Numerous investigators have been able to demonstrate the infectivity of serum or plasma from individuals with acute or chronic non-A, non-B hepatitis.[22,30,33,34] A number of inocula have been pedigreed in this manner and have been passaged several times in susceptible chimpanzees.[35,36] The histologic picture in the liver of non-A, non-B hepatitis virus infected chimpanzees is typical of viral hepatitis.[37] There is no tissue localization of hepatitis A or B virus antigenic markers, but ultrastructural changes, seemingly specific for non-A, non-B hepatitis, have been observed using electron microscopy.[38] Immunofluorescence localization of intranuclear material thought to be specific for non-A, non-B hepatitis has also been observed.[39]

Several serologic tests for a presumed non-A, non-B hepatitis virus antigen have been reported. Techniques to detect viral markers in the serum and liver tissue of humans and chimps with non-A, non-B hepatitis have included gel diffusion, counterelectrophoresis, radioimmunoassay, immunofluorescence, and immune electron microscopy.[33,39–46] Each group of investigators has identified antigenic material in patients with acute non-A, non-B hepatitis that tends to disappear with resolution of the hepatitis or that persists if the infection becomes chronic. In general, the non-A, non-B hepatitis virus tests are negative in pretransfusion specimens or prior to clinical or laboratory evidence of such an infection. In addition, the non-A, non-B hepatitis virus antigen is absent in patients with serologically documented acute hepatitis A or B virus infection. Despite these encouraging aspects, more extensive evaluation must be undertaken before any of the candidate tests can be considered specific for non-A, non-B hepatitis virus infections. Some limited confirmation of non-A, non-B virus associated antigens and antibodies has been achieved,[41] but problems have existed with all these tests in terms of specificity, reproducibility, and confirmability by independent laboratories. A major difficulty has been the general unavailability of sufficient quantities of reagent "antigens" and "antibodies" to exchange among interested laboratories. Overall it would appear that commercially available tests for non-A, non-B hepatitis markers are unlikely in the immediate future.

Several virus particles, in serum or in hepatic tissue, have been associated with non-A, non-B virus hepatitis. A wide range of sizes has been described for these non-A, non-B virus-like particles identified by electron microscopic and immune electron microscopic techniques (Table 1). These size differences may relate to the possible varieties of non-A, non-B hepatitis viruses, differences in methodology, the source and method of demonstration of the virus-like particles, or the unrelatedness of such particles to non-A, non-B infectious agents. Virus-like particles have been observed in known infectious blood products and in the serum of healthy blood donors with elevated alanine aminotransferase levels, although the infectivity of the latter has not been tested.[36,44,46]

With the electron microscope, apparently unique findings have been observed in non-A, non-B virus infected hepatocytes. Two distinct ultrastructural changes have been noted in acute phase liver biopsy specimens of non-A, non-B virus infected chimpanzees.[38] First, virus-like spherical particles were identified in the nuclei of hepatocytes from certain chimpanzees infected with a non-A, non-B hepatitis inoculum (Fig. 1*A*: H). Second, tubular, double membraned structures, which appear to be part of the endoplasmic reticulum, were observed in the cytoplasm of other chimpanzees infected with a different inoculum (Fig. 1*B*: F). The intranuclear particles and the intracytoplasmic tubular structures were not initially observed in the same chimpanzees and seemed to define two distinct non-A, non-B hepatitis containing inocula. These mutually exclusive ultrastructural changes seemed to represent the morphologic manifestations of two distinct non-A, non-B viral agents. Subsequent study has revealed, however, that both findings could occur in the same liver biopsy specimen and that one of the inocula could provoke either nuclear or cytoplasmic ultrastructural change in infected chimpanzees. Limited infectivity and cross challenge experiments in Japan, also performed in chimpanzees, have suggested an immunologic distinction between inocula that regularly produce the intracytoplasmic changes noted on electron microscopy and inocula that do not cause these tubular structures to appear.[47]

In biopsy specimens from humans with chronic active hepatitis thought to be due to non-A, non-B hepatitis, intranuclear particles have been observed but not the cytoplasmic, double walled, tubular structures.[39,48] Hence the significance of the electron microscopic observations of non-A, non-B infected hepatocytes for both man and the chimpanzee, espe-

cially in terms of defining multiple non-A, non-B viral agents, is currently unresolved. What is clear is that both the nuclear and cytoplasmic changes are distinct for non-A, non-B hepatitis and, when visualized, they are very helpful in establishing the diagnosis.

HOW MANY TYPES OF NON-A, NON-B HEPATITIS ARE THERE?

Since non-A, non-B hepatitis is primarily a diagnosis made by exclusion, it has been difficult to discover whether more than one etiologic agent can cause this disease. A number of lines of evidence, however, seem to indicate that two or more viral agents may be responsible. This may vindicate the choice of the nonspecific term, non-A, non-B hepatitis, in preference to hepatitis C.

It was as a result of clinical studies that the existence of more than one agent for non-A, non-B hepatitis was first suspected. Mosley et al.[49] identified three drug abusers with two clear-cut episodes of acute non-A, non-B hepatitis. Craske et al.[50] observed three patients with hemophilia who developed two episodes of non-B hepatitis after infusion with different factor VIII preparations. Earlier, the variability in incubation periods for non-A, non-B hepatitis in transfused patients suggested the existence of more than one non-A, non-B viral agent.[9] Other clinical evidence also favors multiple non-A, non-B agents. The high frequency of chronicity and minimal secondary spread of transfusion related non-A, non-B hepatitis contrasts with the epidemics of water-borne non-A, non-B hepatitis in India where rapid dissemination and minimal rates of residual hepatitis were observed.[20,26]

Chimpanzee studies have been helpful in defining the presence of more than one non-A, non-B hepatitis virus. As already mentioned, the different ultrastructural electron microscopic changes resulting from different non-A, non-B inocula suggested the existence of two viral agents (Fig. 1).[38] Cross challenge infectivity studies in chimpanzees provide more substantive proof of at least two non-A, non-B viruses. Chimpanzees appear to develop immunity after a single exposure to a non-A, non-B inoculum.[51] When they are challenged by a different inoculum, the development of a second episode of non-A, non-B hepatitis should indicate the existence of another agent for this disease. Although most cross challenge experiments have not resulted in second infections,[8,47,51] two groups of investigators have documented second episodes of non-A, non-B hepatitis in four, and in three, chimpanzees.[8,47] Yoshizawa's group[47] also showed that the double walled tubular structures noted in the cytoplasm of infected chimpanzee hepatocytes only occurred with certain inocula; these inocula were cross protective, but inocula that did not result in the cytoplasmic tubules offered no protective effect.

PREVENTION OF NON-A, NON-B HEPATITIS

Effective means to prevent non-A, non-B hepatitis are difficult to establish without specific means for identifying infected individuals, or those already immune. Non-A, non-B hepatitis among drug addicts could be largely prevented if drug paraphernalia were not shared.[13] Sterilization of reusable medical and dental instruments and apparatus, utilization of disposable parenteral materials, proper handling of contaminated needles and syringes, and perhaps isolation of renal dialysis machines used for patients with non-A, non-B hepatitis would obviate some parenterally acquired cases of non-A, non-B hepatitis. In epidemics of non-A, non-B hepatitis, public health measures would appear to be needed to prevent fecal contamination of water supplies.[19,20] It is only in the prevention of transfusion associated non-A, non-B hepatitis that more definitive preventive measures can be undertaken.

Prevention of Transfusion Transmitted Non-A, Non-B Hepatitis

At present several measures can be employed to diminish the risk of non-A, non-B hepatitis transmitted by blood transfusions. The carrier rate for non-A, non-B hepatitis among blood donors has been estimated to be between 1 and 5 per cent. The lower number seems to apply to volunteer donors, whereas the higher number represents that among paid, commercial blood donors.[52]

This knowledge permits the use of two means to decrease the risk of non-A, non-B hepatitis transmitted by transfusions—first, the use of blood solely from volunteer donors and, second, the optimal use of blood and its components. Paid, commercial donors in general carry a higher risk of transmitting viral hepatitis and a higher risk of that transfusion transmitted hepatitis resulting in a fatality.[13] Except for highly specialized or rare blood products, there is no current justification for the use of high risk commercial donor blood. Commercial donors may, however, be used for plasma products, which can be heat treated or otherwise rendered hepatitis free prior to transfusion. In addition, whenever possible, autologous blood and blood products with the lowest risks of hepatitis transmission should be utilized, e.g., the use of single donor plasma or small pools of cryoprecipitated antihemophilic factor in preference to concentrates of clotting factors made from large pools of donors, some of whom are almost certain to be carriers of non-A, non-B hepatitis virus.[52] Lastly, there is a continuing overutilization of blood and blood products. Blood replacement should be limited to the minimal amount required to maintain cardiovascular integrity. Blood utilization, particularly for surgical procedures, should be continuously reviewed. Many hospitals have established blood utilization committees, which have been very helpful in this regard.

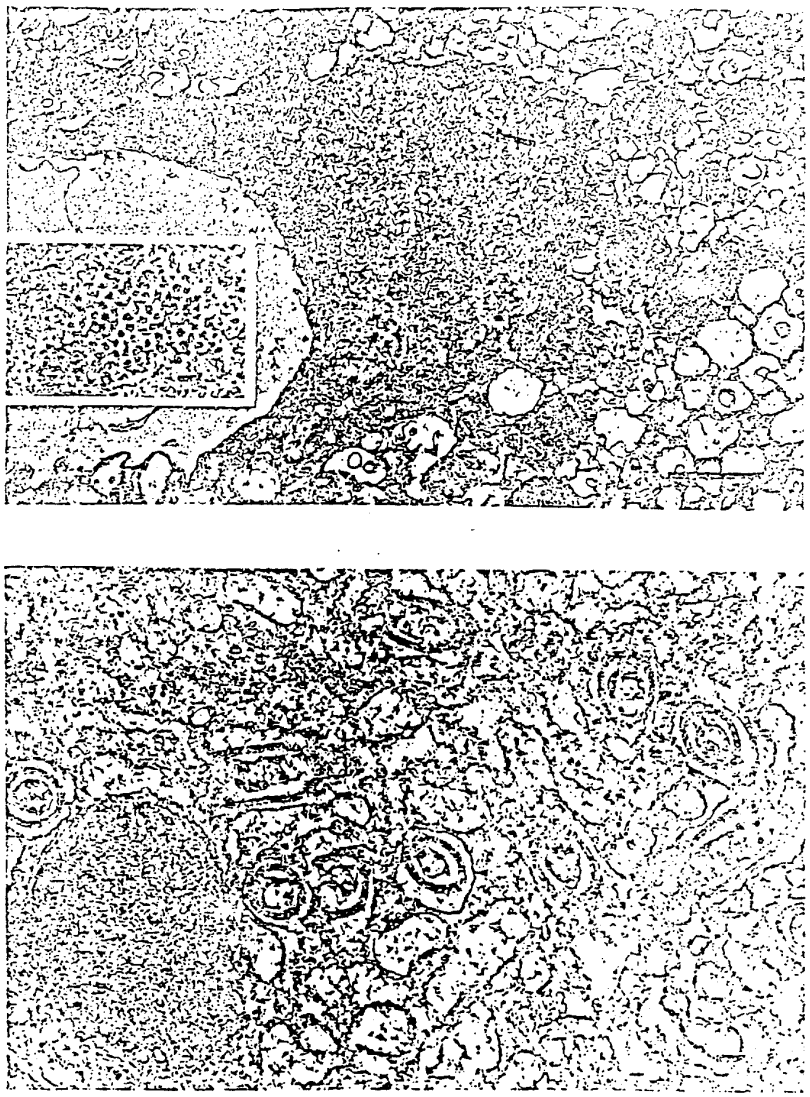

Figure 1. Electron microscopic findings of chimpanzee hepatocytes during infection with non-A, non-B viral agents. The bar represents 1 μm. in *A* but 100 nm. in the inset and in *B*. *A*, Nuclear particles in the hepatocytes of a chimpanzee with non-A, non-B hepatitis six weeks after inoculation with H strain. *B*, Tubular cytoplasmic structures in the hepatocytes from a chimpanzee with chronic non-A, non-B hepatitis after inoculation with F strain.

Cases of hepatitis that occur after transfusions should be diagnosed so that hepatitis implicated donors can be identified. A donor whose blood was the only unit transfused to a patient who developed hepatitis should be permanently excluded from further donations. When hepatitis occurs after multiple units of blood have been transfused, all the donors should be suspected of being the hepatitis virus carrier and their names entered on a donor suspect list. It is impractical to permanently reject all the donors to a multitransfused patient, but implication in more than one case of transfusion hepatitis may warrant permanent exclusion, depending upon the number of donors involved in each case.[54] Unfortunately, non-A, non-B hepatitis can be acquired in hospitalized patients by routes other than transfusion so that some donors may be untairly and incorrectly implicated.[55]

Laboratory Means for Identifying Non-A, Non-B Virus Carriers

Since specific tests for markers of non-A, non-B hepatitis have not been available, nonspecific tests have been utilized in an attempt to identify blood donor carriers of non-A, non-B hepatitis. Various laboratory tests that indicate hepatic dysfunction have

been tried over the years. Most of the early studies were not conclusive.[56] Recently the Transfusion Transmitted Viruses Study group has presented the best evidence to date that measurement of alanine aminotransferase levels in blood donors can lead to a significant reduction in non-A, non-B hepatitis.[57] They estimated that 40 per cent of the cases of non-A, non-B hepatitis transmitted by blood transfusions could be prevented if blood donors with an alanine aminotransferase level higher than 45 I.U. per liter were interdicted from donating; this would result in a loss of 3 per cent of blood donations. These data have been confirmed by our group. Consideration is now being given to the question of whether all blood should be screened for alanine aminotransferase elevations prior to transfusion.

Other nonspecific tests have also been suggested as ways to detect asymptomatic carriers of non-A, non-B viruses. Serum cholylglycine and plasma carcinoembryonic antigen determinations look intriguing as a result of a small study but must be confirmed.[58] In addition, the value of these two tests must be compared with and separated from the use of alanine aminotransferase testing in donors. Since the presence of antibodies to hepatitis B antigens, anti-HBs and anti-HBc, in blood donors may indicate a high risk of exposure to non-A, non-B viruses as well, these measures have been evaluated as means of detecting carriers of non-A, non-B viruses. Studies correlating the non-A, non-B virus infectivity of anti-HBs containing blood have revealed conflicting results.[13,59–61] In the only prospective study evaluating the risk of non-A, non-B hepatitis in recipients of anti-HBc containing blood, non-A, non-B hepatitis occurred significantly more often in recipients of such blood than in recipients of blood without this antibody, regardless of whether the donor blood also contained anti-HBs.[62]

For years the use of frozen blood was advocated to eliminate the risk of post-transfusion hepatitis. This practice was based more on hope and anecdotal observation than on actual clinical study.[63] Finally, a controlled trial to evaluate the hepatitis risk of frozen blood was performed.[64] Four of 104 recipients of previously frozen red cells, recombined with the donors' plasma, developed hepatitis compared to none of 110 recipients of previously frozen red cells combined with heat treated albumin. The small number of hepatitis cases, however, clouds the statistical significance of this study. More recently a careful, but uncontrolled, study has appeared to refute the postulated efficacy of frozen cells to obviate the risk of transfusion transmitted hepatitis,[65] while a new prospective controlled trial of frozen red cells supports their use to diminish the risk of viral hepatitis.[66]

Haugen[63] observed clinical hepatitis in 56 recipients at his hospital who received various blood and blood products from both volunteer and commercial donors; 16 of these patients had received only frozen red cells, 13 had received just washed red cells, and eight had received both frozen and washed red cells. Subsequently Haugen observed a seven-fold drop in the hepatitis frequency at his hospital when the blood bank switched to all volunteer donors. It is clear that a change in donor population had a more important impact on the incidence of hepatitis than the use of frozen or washed red blood cell transfusions.

In an ongoing study Meryman et al.[66] are comparing the risk of hepatitis after the administration of either frozen, just washed, or ordinary packed red blood cells. To date they have seen no cases of viral hepatitis in 43 recipients of frozen red cells, but there have been four cases (one icteric, three anicteric) after transfusion of 72 patients with saline-washed red cells and four cases of hepatitis (three icteric) among 41 patients receiving ordinary packed red cells. Although this trend favors the use of frozen red blood cells, the differences are not significant and conclusions should not be drawn until this study is completed. At best the freeze-thaw-wash process for red cells may diminish the risk of transfusion transmitted hepatitis, but it is not likely to be completely effective in its prevention.

Other Means to Prevent Non-A, Non-B Transfusion-Transmitted Hepatitis

Another means of reducing the hepatitis infectivity of blood products may be ultraviolet irradiation, especially when combined with β-propriolactone.[67] Neither ultraviolet irradiation nor β-propriolactone alone can completely eliminate the hepatitis infectivity of plasma,[68,69] but together they may be quite effective in this regard. With eight susceptible chimpanzees, ordinarily infectious factor IX complex treated with combined β-propriolactone and ultraviolet irradiation was rendered noninfectious for type B and non-A, non-B viruses.[67] Further evaluation of hepatitis B virus material of known infectivity showed that the infectivity for this virus was markedly reduced but not eliminated.[67]

Immune serum globulin has been proposed for the prophylaxis of transfusion associated hepatitis for many years. Many studies have been published on the effectiveness and on the lack of effectiveness of immune serum globulin in the transfusion setting.[70] Most recently the effect of immune serum globulin on non-A, non-B hepatitis caused by transfusions has been evaluated by three groups. Once again there have been conflicting conclusions regarding effectiveness. Kuhns et al.,[71] using immune serum globulin prepared from individuals convalescing from non-A, non-B hepatitis, found no effect on the prevention of transfusion associated hepatitis. On the other hand, Knodell et al.[72,73] concluded that the risk of icteric but not anicteric non-A, non-B hepatitis as well as the frequency of chronicity were both reduced by immune serum globulin. Finally, in their initial study, Seeff et al.[74] noted that immune serum globulin reduced the frequency of icteric cases of non-A, non-B hepatitis, but not that of anicteric cases. This observation, however, was confounded by the large number of high risk commercial donors in this study, and a second study by Seeff et al.[52] did not support this

conclusion of their initial study. Although the latter investigators suggested that immune serum globulin might be appropriate for certain high risk patients, e.g., those receiving three or more units of commercial blood, they pointed out that the elimination of paid, commercial donors would have been the most effective means of reducing transfusion transmitted non-A, non-B hepatitis.

Future Prevention of Non-A, Non-B Hepatitis

The most significant measure for the prevention of transfusion related non-A, non-B hepatitis would be the development of tests to detect specific viral markers. Such serologic tests would allow us to detect carriers of non-A, non-B hepatitis and to determine the susceptibility of exposed patients. Such tests might also aid in the purification of viral antigen for vaccine development along the lines of that found effective with hepatitis B virus.[75] Until such tests are developed and commercially available, nonspecific means of prevention, such as minimal blood usage, an all volunteer blood donor system, recording of implicated donors, and possibly pretransfusion alanine aminotransferase testing, should be utilized.

k owledgments

The authors wish to thank Dr. Hans Denes for providing the electron micrographs of liver tissue showing intranuclear particles and cytoplasmic ultrastructures related to non-A, non-B hepatitis in chimpanzees.

REFERENCES

1. Krugman, S.: Viral hepatitis: overview and historical perspectives. Yale J. Biol. Med., *49*:243, 1976.
2. Gerber, P., et al.: Association of EB-virus infection with the post-perfusion syndrome. Lancet, *1*:593, 1969.
3. A Report to the M.R.C. Blood Transfusion Research Committee by the Medical Research Council Working Party on Post-transfusion Hepatitis: Post-transfusion hepatitis in a London hospital: results of a two-year prospective study. J. Hyg. (Camb.), *73*:173, 1974.
4. Prince, A. M., et al.: Long-incubation post-transfusion hepatitis without serological evidence of exposure to hepatitis-B virus. Lancet, *2*:241, 1974.
5. Feinstone, S. M., et al.: Transfusion associated hepatitis not due to viral hepatitis type A or B. N. Engl. J. Med., *292*:767, 1975.
6. Feinstone, S. M., Kapikian, A. Z., and Purcell, R. H.: Hepatitis A: detection by immune electron microscopy of a ...s-like antigen associated with acute illness. Science, ...:1026, 1973.
7. ...ce, A. M.: Immunologic distinction between I. H. and S. H. N. Engl. J. Med., *281*:163, 1969.
8. Hollinger, F. B., et al.: Transfusion-transmitted viruses study: experimental evidence for two non-A, non-B hepatitis agents. J. Inf. Dis., *142*:400, 1980.
9. Ratzan, K. R., Gregg, M. B., and Hanson, B.: Transfusion-associated hepatitis in the United States: an epidemiologic analysis. Amer. J. Epid., *94*:425, 1971.
10. Blumberg, B. S., Alter, H. J., and Visnich, S. A.: A "new" antigen in leukemia sera. J.A.M.A., *191*:541, 1965.
11. Aach, R. D., and Kahn, R. A.: Post-transfusion hepatitis: current perspectives. Ann. Int. Med., *92*:539, 1980.
12. Title 42, Part 73.775. Fed. Reg., *37*(106):10937, 1972.
13. Goldfield, M., et al.: The consequences of administering blood pretested for HBsAg by third generation techniques: a progress report. Amer. J. Med. Sci., *270*:335, 1975.
14. Hollinger F. B., et al.: HBcAg, anti-HBc, and DNA polymerase activity in transfused recipients followed prospectively. Amer. J. Med. Sci., *270*:343, 1975.
15. Norkrans, G., et al.: Clinical, epidemiological and prognostic aspects of hepatitis "non-A, non-B"; a comparison with hepatitis A and B. Scand. J. Infect. Dis., *11*:259, 1979.
16. Deinstag, J. L., et al.: Etiology of sporadic hepatitis B surface antigen negative hepatitis. Ann. Intern. Med., *87*:1, 1977.
17. Guyer, B., et al.: Non-A, non-B hepatitis among participants in a plasmapheresis stimulation program. J. Inf. Dis., *139*:634, 1979.
18. Villarejos, V. M., et al.: Evidence for viral hepatitis other than type A or type B among persons in Costa Rica. N. Engl. J. Med., *293*:1350, 1975.
19. Wong, D. C., et al.: Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus etiology. Lancet, *2*:876, 1980.
20. Khuroo, M. S.: Study of an epidemic of non-A, non-B hepatitis: possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Amer. J. Med., *68*:818, 1980.
21. Ahtone, J., et al.: Non-A, non-B hepatitis in a nurse after percutaneous needle exposure. Lancet, *1*:1142, 1980.
22. Tabor, E., Seeff, L. B., and Gerety, R. J.: Chronic non-A, non-B hepatitis carrier state: transmissible agent documented in one patient over a six-year period. N. Engl. J. Med., *303*:139, 1980.
23. Perras, S. T., et al.: Lack of transmission of hepatitis non-A, non-B by CPR manikins. N. Engl. J. Med., *302*:118, 1980.
24. Gravelle, C. R., et al.: Hepatitis A: report of a common-source outbreak with recovery of a possible etiologic agent. II. Laboratory studies. J. Inf. Dis., *131*:167, 1975.
25. Khuroo, M. S., et al.: Failure to detect chronic liver disease after epidemic non-A, non-B hepatitis. Lancet, *2*:97, 1980.
26. Alter, H. J.: The dominant role of non-A, non-B in the pathogenesis of post-transfusion hepatitis: a clinical assessment. Clin. Gastroent., *9*:155, 1980.
27. Alter, H. J., Holland, P. V., and Purcell, R. H.: Current status of post-transfusion hepatitis. Pathobiol. Ann., *10*:135, 1980.
28. Feinstone, S. M., and Purcell, R. H.: Non-A, non-B hepatitis. Ann. Rev. Med., *29*:359, 1978.
29. Berman, M. D., et al.: The chronic sequelae of non-A, non-B hepatitis. Ann. Int. Med., *91*:1, 1979.
30. Alter, H. J., et al.: Transmissible agent in non-A, non-B hepatitis. Lancet, *1*:459, 1978.
31. Dienstag, J.: Case Records of the Massachusetts General Hospital. N. Engl. J. Med., *303*:207, 1980.
32. Gupta, D. N., and Sinetana, H. F.: The histopathology of viral hepatitis seen in the Delhi epidemic (1955–1956). Ind. J. Med. Res., *45*(Suppl.):101, 1957.
33. Prince, A. M., et al.: Non-A/non-B hepatitis: identification of a virus specific antigen and antibody. A preliminary report. *In* Vyas, G. N., Cohen, S. N., and Schmid, R. (Editors): Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978, p. 633.
34. Tabor, E., et al.: Transmission of non-A, non-B hepatitis from man to chimpanzee. Lancet, *1*:463, 1978.
35. Tabor, E., et al.: Experimental transmission and passage of human non-A, non-B hepatitis in chimpanzees. *In* Vyas, G. N., Cohen, S. N., and Schmid, R. (Editors): Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978, pp. 419–421.
36. Wyke, R. J., et al.: Transmission of non-A, non-B hepatitis to chimpanzees by factor-IX concentrates after fatal complications in patients with chronic liver disease. Lancet, *1*:520, 1980.
37. Popper, H., et al.: The pathology of viral hepatitis in chimpanzees. Virch. Arch. Path. Histol., *387*:91, 1980.
38. Shimizu, Y. K., et al.: Non-A, non-B hepatitis: ultrastructural evidence for two agents in experimentally infected chimpanzees. Science, *205*:197, 1979.
39. Vitvitski, L., et al.: Detection of virus-associated antigen in serum and liver of patients with non-A, non-B hepatitis. Lancet, *2*:1263, 1979.

40. Shirachi, R., et al.: Hepatitis "C" antigen in non-A, non-B post-transfusion hepatitis. Lancet, *2*:853, 1978.
41. Tabor, E., et al.: Detection of an antigen-antibody system in serum associated with human non-A, non-B hepatitis. J. Med. Virol., *4*:161, 1979.
42. Neurath, A. R., et al.: An antigen detected frequently in human sera with elevated levels of alanine aminotransferase: a potential marker for non-A, non-B hepatitis. J. Gen. Virol., *48*:235, 1980.
43. Kabiri, M., Tabor, E., and Gerety, R. J.: Antigen-antibody system associated with non-A, non-B hepatitis detected by indirect immunofluorescence. Lancet, *2*:221, 1979.
44. Bradley, D. W., Cook, E. H., and Maynard, J. E.: Experimental infection of chimpanzees with antihemophilic (factor VIII) materials: recovery of virus-like particles associated with non-A, non-B hepatitis. J. Med. Virol., *3*:253, 1979.
45. Hantz, O., Vitvitski, L., and Trepo, C.: Non-A, non-B hepatitis: identification of hepatitis-B-like virus particles in serum and liver. J. Med. Virol., *5*:73, 1980.
46. Yoshizawa, H., et al.: Virus-like particles in a plasma fraction (fibrinogen) and in the circulation of apparently healthy blood donors capable of inducing non-A, non-B hepatitis in humans and chimpanzees. Gastroenterology, *79*:512, 1980.
47. Yoshizawa, H., et al.: Demonstration of two different types of non-A/non-B hepatitis by re-injection and cross-challenge studies in chimpanzees. Gastroenterology, *81*:107, 1981.
48. Grimaud, J. A., et al.: Hepatic intranuclear particles in patients with non-A, non-B hepatitis. N. Engl. J. Med., *303*:818, 1980.
49. Mosley, J. W., et al.: Multiple hepatitis viruses in multiple attacks of acute viral hepatitis. N. Engl. J. Med., *296*:75, 1977.
50. Craske, J., Spooner, R. J. D., and Vandervelde, E. M.: Evidence for existence of at least two types of factor-VIII-associated non-B transfusion hepatitis. Lancet, *2*:1051, 1978.
51. Tabor, E., et al.: Acquired immunity to human non-A, non-B hepatitis: cross-challenge of chimpanzees with three infectious human sera. J. Inf. Dis., *140*:789, 1979.
52. Seeff, L. B., et al.: Post-transfusion hepatitis, 1973-1975: a Veterans Administration cooperative study. *In* Vyas, G. N., Cohen, S. N., and Schmid, R. (Editors): Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978, pp. 371-381.
53. Dahmen, E., Malchus, R., and Hoppe, I.: Hepatitis after cardiosurgery. Frequency and causes. Results of a prospective study with use of autologous blood. Thorac. Cardiovasc. Surg., *28*:1, 1980.
54. Walker, R. H., and Inclan, A. P.: Utilization of the biologic test in post-transfusion hepatitis for the detection of carriers. South. Med. J., *58*:1131, 1965.
55. Aach, R. D., et al.: Transfusion-transmitted viruses: interim analysis of hepatitis among transfused and non-transfused patients. *In* Vyas, G. N., Cohen, S. N., and Schmid, R. (Editors): Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978, pp. 383–396.
56. Hollinger, F. B., et al.: Non-A, non-B post-transfusion hepatitis in the United States. *In* Gerety, R. (Editor): Non-A, Non-B Hepatitis. New York, Academic Press, Inc. (In press.)
57. Aach, R. D., et al.: Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients. The Transfusion-transmitted Viruses Study. N. Engl. J. Med., *304*:989, 1981.
58. Gitnick, G. L., Brezina, M. L., and Mullen, R. L.: Application of alanine aminotransferase, carcinoembryonic antigen and cholylglycine levels to the prevention and evaluation of acute and chronic hepatitis. *In* Vyas, G. N., Cohen, S. N., and Schmid, R. (Editors): Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978, pp. 431–438.
59. Aach, R. D., et al.: Risk of transfusing blood containing antibody to hepatitis-B-surface antigen. Lancet, *2*:190, 1974.
60. Seeff, L. B., et al.: VA cooperative study of gamma globulin prophylaxis of post-transfusion hepatitis. Gastroenterology, *64*:893, 1973.
61. Conrad, M. E., et al.: Risk factors in transmission of non-A, non-B post-transfusion hepatitis. The role of hepatitis B antibody in donor blood. Transfusion, *17*:579, 1977.
62. Rakela, J., et al.: Viral hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. Gastroenterology, *78*:1318, 1980.
63. Huggins, C. E.: Frozen blood. Europ. Surg. Res., *1*:3, 1969.
64. Tullis, J. L., et al.: Incidence of post-transfusion hepatitis in previously frozen blood. J.A.M.A., *214*:719, 1970.
65. Haugen, R. K.: Hepatitis after transfusion of frozen red cells and washed red cells. N. Engl. J. Med., *301*:395, 1979.
66. Meryman, H. T., et al.: Post-transfusion hepatitis following transfusion with packed cells, saline washed cells or frozen deglycerolized cells. Presented at ISBT-ISH, Montreal, August 1980 (abstract).
67. Prince, A. M., and Stephen, W.: Effect of combined β-propriolactone ultraviolet irradiation treatment on hepatitis B virus. Lancet, *2*:917, 1980.
68. Murray, R., et al.: Effect of ultraviolet radiation on the infectivity of icterogenic plasma. J.A.M.A., *157*:8, 1955.
69. Barker, L. F., and Murray, R.: Relationship of virus dose to incubation time of clinical hepatitis and time of appearance of hepatitis-associated antigen. Amer. J. Med. Sci., *263*:27, 1971.
70. Holland, P. V.: Gamma globulin in post-transfusion hepatitis. *In* Vyas, G. N., Perkins, H. A., and Schmid, R. (Editors): Hepatitis and Blood Transfusion. New York, Grune & Stratton, Inc., 1972, p. 331.
71. Kuhns, W. J., et al.: A clinical and laboratory evaluation of immune serum globulin from donors with a history of hepatitis. Attempted prevention of post-transfusion hepatitis. Amer. J. Med. Sci., 272:255, 1976.
72. Knodell, R. G., et al.: Efficacy of prophylactic gamma globulin in preventing non-A, non-B post-transfusion hepatitis. Lancet, *1*:557, 1976.
73. Knodell, R. G., Conrad, M. E., and Ishak, K. G.: Development of chronic liver disease after acute non-A, non-B post-transfusion hepatitis: role of gamma-globulin prophylaxis in its prevention. Gastroenterology, 72:902, 1977.
74. Seeff, L. B., et al.: A randomized double-blind controlled trial of the efficacy of immune serum globulin for the prevention of post-transfusion hepatitis. Gastroenterology, 72:111, 1977.
75. Szmuness, W., et al.: Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. N. Engl. J. Med., *303*:833, 1980.
76. Hollinger, F. B., and Alter, H. J.: Summary of workshop B-6; non-A/non-B hepatitis. *In* Vyas, G. N., Cohen, S. N., and Schmid, R. (Editors): Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978, p. 697.
77. Prince, A. M., et al.: Hepatitis C virus (HCV): characterization of virus specific antigens and associated particles. Gastroenterology, 77:A33, 1979.
78. Mori, Y., et al.: Virus-like particles associated with non-A, non-B hepatitis. Lancet, 2:318, 1980.

1E33, Building 10-A
National Institutes of Health
Bethesda, Maryland 20205 (Dr. Holland)