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NON-A, NON-B VIRAL HEPATITIS

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I. INTRODUCTION

Through much of this century, two viruses were believed to account for essentially all cases of infectious jaundice. Not until it became possible to make specific diagnoses of both type A and type B hepatitis was there convincing evidence of additional hepatitis viruses. While the existence of such additional agents can no longer be doubted, there is still uncertainty about how many of these "new" hepatitis viruses there are. Because of this, the rather vague term **non-A, non-B hepatitis** rather than the predictable "hepatitis C", has been applied to the diseases they cause.

With the ability to identify the hepatitis A and B viruses, and to prevent their infections with specific immunization, NANB hepatitis has become even more important because of the continued uncertain identity of its causative agents. Nearly all cases of post-transfusion hepatitis and a significant percentage of community-acquired (sporadic), fulminant and chronic hepatitis cases are caused by these viruses.

This discussion concerns our present understanding of the NANB viruses, the diseases they cause, and prevention of these diseases.

II. THE NANB VIRUSES

The NANB viruses prevalent Western countries behave more like hepatitis B than hepatitis A viruses; they are spread mainly by means of transmission of serum from one person to another, whether by obvious (e.g., transfusion) or more subtle means, they do not appear to spread by the fecal-oral route, and they often lead chronic infection and persistent carrier states.

The most important advance in the study of NANB viruses has been the recognition that the chimpanzee could serve as an animal model of this human disease (1 - 4).

A. Evidence for more than one NANB virus

A considerable amount of data favors the existence of **two or more parenterally-transmitted NANB viruses.** The evidence includes (1) Mosley's observation of two well-defined episodes of hepatitis in each of 3 drug abusers, each episode unrelated to hepatitis A, hepatitis B, cytomegalovirus or E-B virus infection(5); (2) the broad range of incubation periods observed for NANB hepatitis. In the important case of post-transfusion NANB hepatitis (NANB-PTH),

where the time of exposure can be clearly defined, the incubation periods have ranged from 2 to 26 weeks; (3) in some instances, chimpanzees resistant to re-infection on second exposure to the same infectious NANB inoculum did develop evidence of hepatitis after injection with a different inoculum; and, (4) Shimizu (6) described distinctly different patterns of ultrastructural (EM) liver injury in chimpanzees which predictably followed their exposure to two different NANB inocula. All animals receiving one inoculum (NIH strain F) developed cytoplasmic structures in the cisternae of the endoplasmic reticulum. These structures appeared circular in cross section and were composed of two parallel walls when viewed lengthwise. Because of this, this NANB strain is now often referred to as the **tubule forming agent** or NANB-TFA. In the chimpanzees receiving a second inoculum (NIH strain H), the tubular cytoplasmic changes were not seen but, instead, nuclear aggregates of 20 - 27 nm particles, not of a virus-like appearance, were noted. Neither of these two patterns of injury were observed in biopsies from the same animals taken before infection, or in biopsies from chimpanzees infected with type A or B hepatitis viruses.

However, each of these pieces of evidence for separate NANB strains can be questioned. The occurrence of multiple episodes of acute hepatitis in the absence of HAV or HBV markers could, in fact, represent episodic re-activation of a chronic infection with a single NANB viral agent, a phenomenon now well recognized.

The wide range of incubation periods for NANB post-transfusion hepatitis could reflect differences in inoculating dose and host response to a single virus. The incubation period of type B hepatitis, at its extremes, extends from less than one month to over six months.

The chimpanzee cross-challenge studies are confused by observations that, in some instances, re-exposure to the same inoculum which caused earlier infection or to the animal's own serum obtained during the acute phase of his earlier hepatitis, has resulted in renewed transaminase elevations (7, 8).

Further EM study of the same liver biopsy tissue blocks used by Shimizu in his studies describing the distinctive ultrastructural patterns during infection with different NANB virus strains has revealed the presence of cytoplasmic tubular changes in biopsies from those chimpanzees infected with the H strain, originally said to cause only nuclear changes (9). Still, it should be noted that, while their exact relationship to infection with the NANB viruses is unclear, the cytoplasmic tubular change remains a good histologic marker for this infection in chimpanzees (10). These tubular changes have, thus far, been identified in only two human cases; they were present in the liver biopsies of 2 homosexual men with AIDS, one of whom also had chronic non-B hepatitis (11).

Despite the blurring of these apparent discriminators, sufficient evidence remains to lead the reviewer to suspect that more than one virus does, in fact, account for the cases of NANB hepatitis occurring in Western countries. Tabor (12) demonstrated apparent cross-immunity in chimpanzees to inoculation with infectious sera acquired from patients in 3 different areas of the country. It is a reasonable suggestion that however many different NANB agents may exist, one of these, or a small number of serologically related agents accounts for the majority of NANB hepatitis seen in this country. This is a hopeful note, since it may therefore not be necessary to develop assays for numerous agents to prevent most cases of post-transfusion hepatitis.

While certain evidence has led to suggestions that one NANB virus may be an **hepadna virus**, related, at least distantly, to hepatitis B virus, alternative explanations for these findings make this an unlikley possibility (13).

B. Assays for NANB hepatitis viruses

Electron microscopy studies of serum and liver tissue from patients and NANB-infected chimpanzees have led to the description of at least **13 different particulate structures**, ranging in size from 14 to 140 nm in diameter, suspected of representing NANB viruses (10,13). Most consistently identified have been **27 nm particles.**

In addition, over 30 putative NANB virus serologic assays of every type have been described. Many of these were applied to the testing of a panel of coded serum specimens prepared by Dr. Harvey Alter (14). Each specimen, present in duplicate was clearly defined in animal or human transmission studies as being consistently positive or negative for an agent of NANB hepatitis. Of the many putative assays evaluated in this survey, only the RIA of Arnold et al (15) could be shown to be a valid assay. This RIA showed complete agreement for all duplicate pairs, was positive for 68% of the biologically positive specimens, and was negative for all samples proven **not** infectious for NANB hepatitis. In other words, within the confines of this survey, Arnold's assay for NANBV was 68% sensitive, 100% specific and completely reproducible. It has been a great disappointment that this assay, first described years ago, has not fulfilled its initial promise. No furhter reports about it have appeared. Its developers have been unable (unwilling?) to share the reagents of their assay with other investigators, reportedly because of limited reamaining supplies.

A major factor believed to account for the difficulties in

establishing NANB virus assays is the probability that these agents are usually present in the blood in very low concentrations - 10 to 100 infectious doses per ml, typically, as compared with up to 10^7 ID/ml for some HBsAg positive sera. Furthermore, again unlike HBV, the NANB agent(s) is not likely to be "amplified" by a grossly redundant production of antigen comparable to HBsAg. Nor is an NANB agent likely to be present in stool in the very large concentrations occurring with hepatitis A virus infection. In addition, it is possible that NANB viruses circulate "hidden" within immune complexes (16), and that recovery from infection and subsequent development of antibody (for use in detection systems) may occur relatively infrequently, with a majority of infected subjects becoming chronic carriers of the virus (17).

Despite these problems, four promising new assays for NANB virus are now under investigation. Burk et al (8) has described a biotin/avidin amplified immunoperoxidase assay employing as an antibody source serum from chimpanzees convalescent from NANB hepatitis. This antibody was shown to be specific for an antigen present in the cytoplasm of chimpanzees after, but not before, infection with an NANB virus. The staining reaction was specifically blocked by addition of acute phase (presumptive NANBV antigen-containing) serum.

Pastore et al (18) have developed an enzyme-linked immunosorbant assay (ELISA) for NANB virus employing IgG from the serum obtained from a patient 14 months after an episode of NANB post-transfusion hepatitis. Using this antibody both as a "catcher" to bind NANBV antigen to a solid surface and as an enzyme-labeled probe to detect the affixed virus. A NANB antigen was found in 3 of 20 (15%) of patients with NANB post-transfusion hepatitis (NANB-PTH), but in none of a large number and variety of control patients. Furthermore, each of the remaining 17 NANB-PTH patients were shown to have developed antibody to this NANB antigen, and the titer of this antibody rose progressively with the duration of convalescence. (It should be kept in mind that this development of antibody is not be proof that the viral infection had resolved. Rather, the antibody may have been "blocking" detection of persisting viral antigen.)

Seto and associates (19) have taken an imaginative approach to identifying the nucleic acid of a NANB virus. They radiolabeled the total DNA from plasma of chimpanzees acutely infected with NANB virus. When it was shown, without surprise, that this labeled DNA, having "leaked out" of injured liver cells, hybridized extensively with normal chimpanzee liver, these investigators then cloned the circulating DNA in a plasmid. When the recombinant plasmid colonies were screened with host DNA, one plasmid, containing a small (720 base) insert, was identified as able to hybridize with DNA in NANB-infected but not normal

chimpanzee liver. Further information on these studies is awaited with great interest.

Finally, and most recently (63), Gerety and associates at the FDA report finding particle-associated reverse transcriptase activity in six different specimens each previously shown to transmit NANB hepatitis to human subjects and/or chimpanzees. The sucrose density gradient fraction with maxiaml enzyme activity transmitted NANB hepatitis to chimpanzees. This reverse transcriptase activity is an indication that one or more NANB agents may be retroviruses. Heretofore, the only recognized human retroviruses have been the HTLV group.

III.THE DISEASES CAUSED BY NANB VIRUSES

It is estimated that prior to the availability of tests to screen blood donors for hepatitis B virus (HBV) about one fourth of post-transfusion hepatitis cases were due to this agent (61). Now that such screening is routine, HBV is the cause of only 2 - 10% of transfusion-associated hepatitis cases. Despite a rather small reduction in the total incidence of PTH following the institution of HBsAg screening, it appears that acute type B hepatitis was, on average, a considerably more severe disease than that due to NANB virus(es) as indicated by the observation that PTH mortality dropped to one eighth the previous level after establishment of routine HBV screening (62).

The **clinical features** of acute NANB hepatitis do not distinguish this disease from types A or B hepatitis in an individual case. On average, however, NANB cases are **milder**, a majority (75%) being **anicteric**, with few (about 10%) having maximal transaminase levels over 2000 IU/L (20,21). Many cases are **asymptomatic**, even when the disease has progressed to destructive chronic hepatitis or even cirrhosis.

Shirachi (22) noted three transaminase patterns among patients with acute NANB infections; half had a monophasic elevation, one fourth displayed a biphasic pattern, and the remainder showed a plateau of mild ALT elevations which gradually returned to baseline values. At present there is little evidence to suggest that these patterns reflect infection with different NANB viral strains.

A number of reports have emphasized the widely **fluctuating aminotransferase (ALT) values** occurring in patients with chronic NANB infections. It is important to recognize that one aspect of this pattern may be intervals (days to weeks) of **normal transaminase values** bracketed by elevated values (23). This pattern is quite different from the usual behavior of transaminases in chronic type B hepatitis; in that case, normalization of ALT values usually provides reassurance

that the disease has become less active and may, in fact, have subsided completely. This may be further supported by seroconversion from e-antigen (HBeAg) to anti-HBe, and even by reversion to negative of the HBsAg test; thereafter, "relapses" of chronic hepatitis B are infrequent.

Clearly, in the case of chronic NANB hepatitis, one or a few normal transaminase determinations may not be so reassuring as in hepatitis B patients. There are few data on the histologic (liver biopsy) activity of chronic NANB hepatitis during these "silent intervals" of normal transaminases, but there is reason to believe that, at times, active liver injury is proceeding during these intervals. This phenomenon also complicates interpretation of the response to any treatment efforts; in addition, it may obscure the progression of acute NANB hepatitis to a clinically inapparent chronic infection, if such progression is looked for with a single transaminase determination 6 months or so after onset of illness. Among blood donors, the majority subset of NANB carriers who are not identified by ALT screening may include a number of persons with chronic NANB hepatitis whose transaminase values are elevated at other times. Finally, if normalization of ALT values after acute NANB hepatitis is (mis)interpreted as resolution of the disease, subsequent recurrent elevations may be mistakenly attributed to other viral or non-viral factors.

By light microscopy, the **histology of NANB hepatitis** does not allow its distinction from that due to other hepatitis viruses. But Dienes and Popper (24) have noted somewhat unique changes in the liver biopsies of about half of the NANB patients they studied. These included (1)**eosinophilic clumping** of the cytoplasm, (2) small-droplet **fatty infiltration** which was not otherwise explained, (3) marked proliferation of **sinusoidal lining cells**, (4) a lesser degree of **inflammatory response** (lymphocytic infiltration) than seen with types A and B hepatitis, and (5) an unusually large number of **acidophile bodies.** In the NIH Clinical Center series of NANB hepatitis cases, 17 of 25 showed fatty changes on liver biopsy, which in some cases was quite marked (25).

The relative lack of parenchymal lymphocytic infiltration has suggested that liver cell injury associated with NANB infection may be the result of a **direct cytopathic effect** of the virus rather than to the host's immune response to that virus as believed to be the case in HBV infection. But, somewhat contrary to this view, is the finding in Bradley's studies (26) that cyclophosphamide treatment of a NANBV-infected chimpanzee led to normal ALT levels despite massive proliferation of hepatocyte endoplasmic reticulum, presumably related to the cytoplasmic tubular changes discussed above?).

A. Parenterally-transmitted hepatitis

The NANB viruses are transmitted under the same conditions as hepatitis B virus - i.e., by blood transfusion, accidental needle-stick, parenteral drug abuse, and among hemodialysis and renal transplant patients, health care workers, from infected women to their newborn infants at or before birth, etc. The major issue of NANB post-transfusion hepatitis is considered in greater detail in the section in the Prevention section.

B. Sporadic NANB hepatitis

In a distillation of several series, it appeared that sporadic (i.e., apparently non-parenterally transmitted; "community-acquired") acute NANB hepatitis represented 6 - 18% of cases, as compared with hepatitis A, 10 - 57% and hepatitis B, 34 - 74% (27). In a CDC survey (28) monitoring acute hepatitis incidence in 5 sentinel counties, it was observed that overall, 26% were NANB, 41% type A and 33% type B. NANB hepatitis occurred equally in both sexes, relatively more often in persons over age 40, and without seasonal fluctuation in incidence. **Risk factors** identified included parenteral drug abuse, transfusion history, previous contact with jaundiced persons, and previous hospitalization, independant of transfusion.

In the CDC survey, **homosexuality** was not identified as a risk factor. Szmuness (29) noted a 2.9% annual incidence of NANB hepatitis in gay men, as compared with 5.2% for hepatitis A and 18% for hepatitis B. Whether this NANB incidence is higher than in a closely comparable straight population is uncertain, but it is clear that in this group, NANB virus is less likely to be transmitted than the other hepatitis viruses. This may be a reflection of the relatively low viral concentrations in the blood of NANBV infected persons.

Person-to-person spread of NANBV has been reported within **families** (30) and in institutions for the **mentally retarded** (31). Nevertheless, such transmission occurs rather infrequently. NANB appears to be the least contagious of the the 3 major hepatitis viruses.

Hellings (32) failed to demonstrate hepatitis transmission to chimpanzees when inocula of either of 2 NANB strains (F and DS, which may be the same) were administered **orally**, while hepatitis resulted from subsequent intravenous injection of the same inocula.

A number of cases of **fulminant** NANB hepatitis have been reported.

Among a total of 203 fulminant cases collected from several different reports, Dienstag observed that NANB infection accounted for 27 -44% of cases. Mortality (87 -100%) was higher than for types A or B fulminant hepatitis (33). In notable contrast, **fulminant cases have not been reported among patients with post-transfusion NANB hepatitis** . This suggests that the fulminant sporadic cases may be of a different viral or non-viral etiology; for example, it is probable that at least some of the presumed NANB fulminant cases reported were, in fact, due to HBV in patients who had become HBsAg negative by the time they were first tested for this marker. (Such rapid disappearance of HBsAg in fulminant cases is well known.) (20). Gimson (34) reported that in London, NANB infection was the most common type (43.8%) among 75 patients with fulminant hepatitis, followed by type A hepatitis in 31.5% and type B in 24.7%. The NANB cases had the **lowest survival** - 3 of 33 (9.3%) as compared with 16.6% for type B and 43.4% for type A hepatitis.

Papaevangelou (35) determined the etiology of 65 cases of fulminant hepatitis seen in Athens between May 1981 and August 1983. NANBV was the apparent cause of 16/65 (24.6%) cases, as compared with 48 (73.9%) due to HBV and one due to HAV infection. As has become common practice, these authors used the presence of IgM hepatitis B core antibody (IgM anti-HBc) as the criterion for acute type infection, assuming that in HBsAg (+) patients without the IgM antibody, the acute illness was due to another factor in a previously unrecognized HBV carrier. By these criteria, it appeared that in ten cases **fullminant hepatitis was due to NANB infection of chronic HBV carriers.** However, the authors conceded that in these cases, non-viral liver injury or exacerbation of chronic type B liver disease could not be ruled out. Nevertheless, they tentatively concluded that chronic HBV carriers may be at increased risk of severe superinfection by NANB viruses as they appear to be from infections with the delta agent and, perhaps, with hepatitis A virus.

C.Chronic NANB hepatitis

Post-transfusion NANB hepatitis has consistently shown a remarkable propensity **to progress to chronic hepatitis.** In a group of 16 reports of collectively involving a total of 747 NANB post-transfusion hepatitis cases, 330 (44%) continued to have ALT elevations 6 months or more after onset, the usual criterion for progression to chronic hepatitis (33). In 5 of these reports, type B post-transfusion hepatitis was also considered; chronic hepatitis occurred in 126 of 277 cases (45%) of NANB infections. While the commonly cited figure for progression of acute type B hepatitis to chronicity is 5 to 10% (64), in these 5 reports, 22% of type B cases became chronic (33). Nevertheless, if these

combined data can be compared statistically (which is questionable), the likelihood of NANB post-transfusion hepatitis going on to chronic disease is still significantly greater ($p<<0.0001$).
Chronic hepatitis has also developed in NANBV-infected chimpanzees, though not with the same frequency or severity as in humans (16). In 3 of 5 chronically infected chimpanzees, the liver biopsy revealed a pattern of chronic persistent (i.e., benign) hepatitis (36). Chronic active hepatitis and progression to cirrhosis have not been reported in chimpanzees.

Although it is evident that "silent" NANB carriers (asymptomatic, with normal ALT levels) are several times more common than HBV carriers, at present there are insufficient data to know how many of these NANB carriers have chronic hepatitis and how many are truly "healthy carriers" with normal liver histology. Bradley (36) has demonstrated the persistence of morphologic lesions in chimpanzees, even after ALT levels have become normal.

On the basis of two earlier reports, it has generally been thought that **sporadic NANB hepatitis was less inclined to become chronic than transfusion- associated NANB infections;** Rakela reported 20% chronicity after sporadic NANB hepatitis in Los Angeles (37), and Norkrans observed a rate of 7% chronicity in Scandanavian cases (38). However, two other studies have each shown a 42% rate of progression after sporadic infections in Baltimore (39), and in Great Britain (40).

In either sporadic or transfusion-associated cases, the frequency of progression to chronic disease could be falsely reduced by the inclusion with NANB group of cases due to toxic factors or viruses not prone to cause chronic infections. As an example of the latter situation, Alter et al (14) found, in a survey conducted at the NIH Clinical Center during 1978 and 1979, that 15% of cases meeting the usual definitions of NANB-PTH were, in fact, probably due to cytomegalovirus infection (a rate of CMV infection which, curiously, has dropped to zero in that institution since then (10)). Since none of the CMV post-transfusion hepatitis cases became chronic, the rate of chronic hepatitis after "true" NANB infection could be raised to 65% from the previous figure of 50% (14).

A disturbing feature of chronic NANB-PTH is that the majority of patients are found on liver biopsy to have developed **chronic active hepatitis** (CAH) rather than the more benign chronic persistent hepatitis pattern. Despite the infrequency of the more threatening histologic features of CAH such as bridging necrosis and multilobular collapse, and the usual absence of symptoms, chronic NANB-PTH has been reported to progress "silently" to **cirrhosis** in approximately 10 to 20% of cases. Deaths due to **liver failure** have been observed in several series (37,41-44).

Cirrhosis has developed as early as 4 months after onset of hepatitis (45), and within a year in several other cases.

While the ultimate development of **hepatocellular carcinoma** has been attributed to chronic NANBV infection (as it is known to be a complication of chronic HBV infection) (46,47), knowing the real significance of this association awaits the availability of a specific NANB virus assay, and perhaps especially, identification of its nucleic acid; the latter may be accomplished soon, as discussed above.

Immunosuppressive **therapy** of chronic NANB hepatitis has been apparently beneficial in some series (42,48), but not in others (49). The final judgment as to whether any therapy benefits chronic NANB hepatitis must be deferred until specific assays for the virus, and perhaps more specifically, for its nucleic acid, are developed, and there is better understanding of the natural history of these diseases.

D. Epidemic NANB hepatitis

In recent decades several large outbreaks of **water-borne hepatitis** have occurred in India and neighboring countries. These had been attributed to intensive exposure to hepatitis A virus, adults being more prone to infection presumably because of their waning immunity to HAV. The best known of these outbreaks occurred in Delhi in 1955. This epidemic arose from sewage contamination of the public drinking water supply (50); 29,300 cases were recorded. Recent analysis of sera collected during the Delhi outbreak demonstrated that HAV was not involved in its etiology (51) - i.e., the specimens were all negative for IgM anti-HAV which is believed to be a highly reliable indicator of acute type A infection. It is concluded, therefore, that a water-borne NANB agent is the cause of these epidemic infections. It could be speculated that the relative resistance of children to infection with this virus is evidence of its antigenic relationship to HAV, since children are likely to have greater immunity to the latter virus because of their more recent history of HAV infection.

Epidemic NANB hepatitis is distinctive in the high percentage of infected patients showing **cholestatic features**, both clinically and on liver biopsy, by the **severity** of the illness, particularly in pregnant women and leading to high mortality in this group, and finally, like type A hepatitis, lack of progression to chronicity (52).

As Papaevangelou (35) had shown in Athens for a presumed "Western" type of NANB virus, Tandon (Lancet 9/29/84; letter) has

reported that in an Indian study 17 of 21 (80.9%) of HBsAg(+) patients with **fulminant hepatitis** were negative for IgM anti-HBc, again suggesting the possibility that **superimposed NANB infection**, of the water-borne variety in this case, in a chronic HBV carrier may lead to severe acute liver injury. Tandon has discussed evidence for **two epidemic NANB hepatitis viruses**, one causing a mild form of hepatitis and the other causing severe disease with high mortality, especially in pregnant women.

Kane (53) has apparently **identified the water-borne NANB hepatitis virus**. He has isolated a 27 nm virus-like particle from the stool of infected patients, used this preparation to infect marmosets (New World monkeys), and subsequently isolated the same particles from the stools of the infected animals. Serum from convalescent marmosets agglutinated these particles on immune electron microscopy.

V. PREVENTION OF NANB HEPATITIS

A. General

The CDC has offered no specific guidelines for the prevention of NANB hepatitis after presumed exposure. Certain studies have provided suggestive evidence that Normal Human Immune Globulin (ordinary gamma globulin) might reduce the severity of NANB post-transfusion hepatitis, and perhaps, its likelihood of going on to chronic hepatitis (54,55), but in subsequent studies one of these authors could not confirm such a protective benefit (56). Gerety (57) was unsuccessful in an attempt to prevent NANB infection of chimpanzees using gamma globulin, even when it was mixed with the infectious inoculum prior to injection.

It should be noted that there is a report of the transmission of NANB hepatitis by gamma globulin prepared for intravenous administration (Lane, Lancet 10/22/1983; letter).

It has been demonstrated that at least some NANB viral strains con be **inactivated** by the **heat** treatment ($60^{o}C$ for 10 hours) used for "sterilization" of heat-tolerant plasma derivatives, **formalin** 1:2000 for 36 hours, and **chloroform** extraction (13).

The prospect for a **NANB vaccine** would seem remote at present, but the possibility of vaccination is suggested by the experience of Gerity (57) who demonstrated that one NANB (+) serum sample (Inoculum I) could be made non-infectious to infant chimpanzees by heating it under the conditions noted above. Subsequent intravenous administration of the active (unheated) inoculum to the same animals resulted in only mild infection in one chimpanzees and none in the other.

B. Prevention of Post-transfusion hepatitis

The **incidence** of NANB hepatitis following transfusion of volunteer donor blood ranges from 4.8 to 12.8% (average 7.0%) of transfusion recipients, as compared with an average incidence of 28% after receipt of one or more units from commercial donors (57).

The NANB virus **carrier rate** ranges from 1 - 2% in volunteer donor populations to perhaps 5% or more among paid donors (56,58). Surely a most urgent priority is the development of specific NANB virus assays for the identification of such carriers among blood donors; the difficulties and promises in establishing such assays are discussed above. Until this is accomplished, other ways of preventing NANB post-transfusion hepatitis must be used. In order of diminishing importance, these include avoidance of commercial (paid donor) blood for all blood products which cannot be heat-treated, minimizing blood transfusion, use of frozen/deglycerolized RBCs, and autologous blood transfusion (the use, usually for elective surgery, of the patient's own blood which had been drawn previously and stored frozen; expensive).

Another important consideration, in the absence of specific NANB assays, is the use of "surrogate" tests as indirect indicators of potential NANB virus carriers. Most important among these is screening for elevations of **ALT** (alanine aminotransferase; formerly, SGPT).

Two prospective controlled studies each provided convincing evidence that the risk of post-transfusion NANB hepatitis was closely correlated with the receipt of blood from donors with increased ALT levels (58,59). In the larger of these studies (58) 1513 transfusion recipients were prospectively evaluated for development of post-transfusion hepatitis. It was shown that their hepatitis incidence was directly related to the maximum ALT level among the blood units received. Remarkably, this effect became evident at ALT levels as low as 1/3 the upper limit of normal (ULN) - i. e., there is greater risk of NANB hepatitis after transfusion of blood with an ALT level of 30 than of 15 IU/L (when the ULN is 45 IU/L). The smaller study, which was conducted at the NIH Clinical Center, came to essentially the same conclusion (59); these investigators estimated that by using a cut-off calculated to exclude blood from the 1.6% of donors having the highest ALT levels, NANB post-transfusion hepatitis might be reduced by 30%.

Despite these impressive data, advisory committees of the major blood bank organizations (the American National Red Cross and the American Association of Blood Banks) have, thus far, recommended against routine ALT screening of donors. This judgment has been based primarily on the conclusion that the anticipated benefit of ALT screening, yet unproven in prospective studies, is insufficient to

justify excluding perhaps 3 to 6% of the donor population, particularly considering that 60% of those excluded would have ALT elevations due to non-viral factors bearing no risk to the blood recipient.

Nevertheless, ALT screening has been performed routinely in certain areas of the country (New York City, Indianapolis, NIH) for the past few years. No prospective, controlled trials of donor ALT screening have been carried out (on ethical grounds, the NIH Institutional Review Board rejected a proposal for such a trial). The only data available on the effect of ALT screening are from the NIH Clinical Center (10). There the incidence of NANB post-transfusion hepatitis during three years before screening began was compared with the incidence during the first three years of its utilization. These investigators, who had conducted one of the two trials suggesting the benefit of ALT screening, were surprised to find that the exclusion of all blood units with ALT values over 50 IU/L (thereby rejecting 1.6% of donors) led to no evident decrease in the incidence of post-transfusion hepatitis.

The two large trials also provided clear evidence that NANB carriers may (at least part of the time) have normal ALT levels, and so escape detection by transaminase screening. This has been demonstrated in an individual case where the blood of a chronic NANB virus carrier continued to be infectious for chimpanzees even after his ALT level had returned to normal (60).

SUMMARY

- Probably at least two different viruses are the cause of NANB hepatitis occurring in Western countries.
- It is suspected that one of these agents accounts for the majority NANB infections.
- Another NANB agent, the cause of major epidemics of hepatitis A - like infection in India, has now been specifically identified.
- A major source of morbidity among transfused patients is hepatitis caused by the blood of donors who are unrecognized NANB virus carriers.
- These carriers are several times more common than HBV carriers.
- Despite many unsuccessful attempts, the prospects for development of specific NANB virus assays in the near future are promising.

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