

Review Article

Parenterally acquired non-A non-B hepatitis ten years on: advances in diagnosis and therapy

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Historical perspectives

It has been realized for more than fifty years that patients receiving blood transfusions are at risk of developing hepatitis.¹ Attention to the possible causes of hepatic injury associated with blood transfusion excluded non-infectious problems, such as hepatic congestion, iron overload or chemical contaminants. Further studies in humans and animals soon confirmed that the hepatitis was due to a filterable infectious agent, too small to be anything larger than a virus.² Serological tests for the viruses causing hepatitis B were developed in 1965 and for hepatitis A in 1973, and it was then realized that post-transfusion hepatitis was not related to either of these agents, or to any other known viruses.³ At this time, the term 'non-A non-B' hepatitis was first coined in an attempt to label this presumed hepatic infection.

Identifying the agent responsible so that serological tests can be developed to screen blood and blood products for the presence of this agent is of prime importance, as non-A non-B (NANB) hepatitis is a major cause of morbidity. Acute fulminant NANB hepatitis has a higher fatality rate than any other form of viral hepatitis,⁴ and it has also been estimated that between 40–50% of patients acquiring NANB infection will develop chronic hepatitis, with 15–20% of these subjects progressing to cirrhosis.⁵ It has been estimated that in the USA approximately 6000 patients per annum develop cirrhosis as a direct consequence of chronic NANB hepatitis.⁶

For the past decade, many groups of workers have endeavoured to identify the agent(s) responsible for this disease. In spite of the spectacular advances in molecular biology which have paralleled the same period, it is only within the past 2 years

that a breakthrough has occurred. Part of the genome of an RNA virus (related to the toga- and flaviviridae) that is responsible for many, though not all, cases of NANB infection has been sequenced. This has been labelled hepatitis C virus, and studies indicate that it is responsible for approximately 70–95% of cases of parenterally transmitted non-A non-B hepatitis. This still leaves a significant proportion of cases presumed due to another NANB agent. Within the past year molecular biologists in the USA have also cloned a different RNA virus which is believed to be responsible for the most cases of enterally transmitted NANB infections, and has been provisionally termed hepatitis E.^{6a}

Although identification and characterization of the NANB agents is not yet complete, attempts have still been made to treat this disease, in view of the high morbidity associated with this disease. Recent trials of alpha-interferons have given very encouraging results and hold out hope for a significant improvement in the prognosis of affected individuals. This review looks at recent studies on the nature of this disease, the latest attempts at isolating the causative agent(s), and, lastly, attempts to suppress and/or eliminate this infection.

Nature of the agent

Pathophysiology

Unlike hepatitis A and B infection, the NANB agent(s) are probably cytopathic, causing liver damage by direct lysis of hepatocytes.⁷ In hepatitis A and B infection, there is immunologically mediated hepatocyte injury, both from the humoral and cellular arms of the immune system.^{8,9} In contrast, studies on hepatic autoreactivity in NANB infection indicate that it is unlikely that autoimmunity plays a significant role in mediating hepatocyte damage.¹⁰ In support of this, histological studies have shown that there is in general a paucity of

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intrahepatic lymphocytes in NANB infection compared to hepatitis A and B infection.⁷ Further support comes from studies on the effects of interferon treatment in chronic NANB hepatitis, where there is a fairly prompt fall in aminotransferase levels after interferon therapy is started.¹¹ This is in contrast to hepatitis B infection, where aminotransferase levels remain elevated for some time after therapy is started, until a late rise in enzyme levels several weeks later indicates immune mediated lysis of infected hepatocytes.¹²

How many agents?

The discovery of the hepatitis C virus (HCV) and the availability of antibody tests against HCV antigens and the exquisitely sensitive polymerase chain reaction for detecting HCV nucleic acid in tissues,¹³ has allowed detailed study of patients with chronic NANB hepatitis. It is now apparent that about 20% of patients with chronic NANB hepatitis have no detectable antibodies to HCV, or HCV nucleic acids detectable within the liver.¹³ This suggests that there may be two agents responsible for NANB hepatitis; the first is hepatitis C, which is responsible for most cases of parenteral NANB infection, and the second is a non-A, non-B, non-C (NANBNC) agent, responsible for the remainder. However, this had been predicted several years ago, on the basis of physicochemical studies which strongly suggested the existence of two distinct infectious agents; one that could pass through an 80-nm membrane filter, was sensitive to chloroform (indicating a lipid-containing envelope) and formed characteristic cytoplasmic tubules; and a second agent which was resistant to chloroform and did not induce tubule formation.¹⁴ Earlier studies on the incubation-periods of NANB hepatitis, also suggested that there may be two types, one with short (1–4 weeks) and a second with long (6–10 weeks) incubation periods.¹ Cross challenge experiments in chimpanzees have shown that it is possible to infect animals sequentially with both of these agents, suggesting that these two agents are immunologically distinct.¹⁵ Haemophiliacs are more likely to develop 'short incubation' NANB hepatitis suggesting that factor VIII concentrate is the carrier for this agent,¹⁶ whereas blood transfusion recipients are more likely to get 'long-incubation' NANB suggesting that whole blood is the source of this agent.³ However, as both factor VIII and whole blood are derived from the same heterogenous pool of donors, it is somewhat surprising that the short incubation agent should be associated solely with factor VIII, and the short incubation period, rather than indicating a separate NANB agent, may instead be related to the recognized immunological abnormalities seen in many haemophiliacs,¹⁷ allowing the faster onset of

hepatitis. Further confusion comes from studies showing that short incubation and long incubation disease can be caused in the same animal by increasing the dose of inoculum.¹⁸ At present, the balance of available data supports the existence of two discrete parenterally transmitted NANB agents, but further studies are needed to confirm the existence of the second NANBNC agent.

Possible candidates

Togaviruses

Recent exciting work has indicated that an RNA virus that may be related to the togaviridae or flaviviridae families is likely to be the cause of a large proportion of parenterally transmitted NANB hepatitis cases.^{19,20} Initial suspicion that a small, lipid-enveloped RNA virus was responsible came from studies by Bradley and colleagues in the USA, who performed extensive physicochemical studies on the nature of the NANB agent(s) and found that one of the types is sensitive to chloroform (indicating a lipid-containing envelope) and will pass through an 80-nm membrane filter.^{13,21} The togaviruses (which cause dengue and yellow fever and various types of encephalitis) are a group of enveloped RNA viruses which have the same physicochemical properties as the agent described, and induce similar cytoplasmic and ultrastructural changes.²¹ Confirmation that an RNA virus is responsible for many cases of NANB hepatitis has recently come from the Chiron Corporation in the USA, who recently constructed a complementary DNA (cDNA) library by random-priming of plasma derived from a chimpanzee containing a relatively high infectious titre of NANB virus.¹⁹ A cDNA clone was isolated by screening the clones with antibody to the virus, using serum obtained from a chronic NANB hepatitis patient as a presumed source of antibody. This cDNA clone was shown to encode an antigen associated specifically with NANB infections, and sequencing indicates that it appears to be related to the togaviridae or flaviviridae class of viruses. Using a yeast expression system to produce viral protein from this cDNA clone has allowed the detection of antibody in the sera of patients with NANB hepatitis.²⁰ Termed hepatitis C virus (HCV), preliminary reports indicate that 70–80% of patients in the USA, Japan and Italy with parenterally-acquired chronic NANB hepatitis have antibodies to this virus.²⁰ In addition, about 60% of patients with no history of parenteral exposure (so-called 'sporadic' NANB hepatitis) also have antibodies to HCV. As well as antibodies to the virus, HCV nucleic acid sequences have also been detected in the livers of patients with chronic

NANB hepatitis,¹³ indicating continuing viraemia in patients with chronic infection. Many studies have now reported on the incidence of antibodies to HCV (anti-HCV) in normal individuals and various disease states. The incidence in apparently healthy UK blood donors is between 0.5 and 1%.²² Unfortunately, there appears to be a relatively high incidence of falsely positive anti-HCV tests, particularly in patients with high serum globulin levels, which are often seen in patients with cirrhosis, and autoimmune liver disease particularly.²³ Anti-HCV is also rarely found in the early phase of NANB hepatitis and does not become detectable until 20 weeks after infection.²⁴ Thus the anti-HCV test at present will be unlikely to help in the differential diagnosis of acute viral hepatitis. Nevertheless, the anti-HCV test should prove extremely useful in patients with chronic NANB hepatitis, whether sporadic or parenterally transmitted.

Retroviruses

Much excitement occurred when reverse transcriptase activity was detected in some sera known to transmit NANB infection.²⁵ This suggested that a retrovirus or retrovirus-like agent might be the cause of some cases of infection. A more recent study found reverse transcriptase activity present in the sera of only 9% of patients with NANB hepatitis.²⁶ In light of the evidence for the existence of a second NANB agent, it is possible that a retrovirus or retrovirus-like agent may be responsible for a proportion of the remaining cases of NANBNC hepatitis. Some additional support for the retroviral theory has come from ultrastructural studies showing alterations in the cytoplasm of both hepatocytes and lymphocytes in chronic NANB hepatitis, which are similar to those found in other known retroviral infections.²⁷ These results also suggest that the NANB agent may reside in lymphocytes in the blood, and indeed it has been shown that isolated, washed mononuclear cells from infected patients are infectious and transmit the disease.²⁸ The presence of the agent in lymphocytes is an attractive proposition as it may explain the problems in attempting to isolate the virus from sera. If a lymphocyte-associated retrovirus is indeed the cause of some cases of NANBNC hepatitis, then future studies concentrating on the lymphocytes of infected individuals may be useful. Interestingly, hepatitis B virus, although not a retrovirus *per se*, shares certain characteristics with retroviruses, utilizing reverse transcriptase to synthesize DNA from an RNA genome.²⁹ In addition, hepatitis B virus DNA has also been isolated from the lymphocytes of patients with chronic hepatitis B infection.³⁰

Treatment

Although the agent responsible for NANB hepatitis has been evasive for so long and even though its identity is still far from clear, there seems to be some hope for an effective treatment for this infection in the form of alpha-interferon.

Interferons are proteins that have antiviral activity in many different cells against a wide variety of viruses. The antiviral effects are still not fully understood but involve the induction of intracellular RNAases which degrade viral RNA, and also protein kinases which inhibit viral protein synthesis.³¹ Alpha-interferon also increases MHC expression at the cell surface (allowing recognition of virus-infected cells by cytotoxic T lymphocytes) and also stimulates the cytotoxic T cells to kill these infected cells.³¹ Alpha-interferon is effective in some patients with chronic hepatitis B infection¹² and is well tolerated by patients with no major side-effects, although minor 'flu-like' symptoms are common.

Alpha-interferons have now been studied in chronic NANB hepatitis, and two initial uncontrolled studies in patients^{11,32} have indicated encouraging results with aminotransferase levels returning to normal within 2 months of therapy in approximately 80% of patients, and histological improvement being demonstrated in some subjects after a year's continuous therapy.¹¹ Randomized controlled studies have now been performed in several centres, and results confirm the earlier findings of rapid normalization of aminotransferase levels whilst on low-dose interferon in 50–70% of patients.^{33–35} In parallel with the biochemical improvement, there is also histological improvement with suppression of hepatocellular degeneration and reduction of inflammation in treated patients.^{34–36} Non-responders to interferon are more likely to have a more severe liver disease,³⁷ and higher doses of interferon used for longer periods of time may be more successful in this group of patients. Of course the long-term goal is a permanent 'cure', with normalization of aminotransferase levels and histology even after treatment has been discontinued. One study has indicated that sustained remissions may be achieved in up to 50% of patients who respond to interferon therapy and who are treated for 6 months continuously.³⁴ However, even if relapse does occur on stopping therapy, continuous low-dose interferon is reasonably well tolerated by patients and is an acceptable inconvenience in view of the high risk of development of cirrhosis.

Conclusion

Over 10 years after the term 'Non-A, non-B

hepatitis' was first used, there have been considerable advances in the aetiology and treatment of this disease. After much intensive research, one candidate organism has at last been isolated which may explain many cases of both sporadic and parenterally-transmitted chronic NANB hepatitis. The encouraging results obtained with alpha-

interferons lead us to the situation of being able to treat many patients with this infection successfully without knowing exactly the true nature of the infective agent. The characterization of the NANB and NANBNC agent(s) will continue and, hopefully, lead to the development of specific serological tests for screening blood and blood-products.

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