The Serology of Hepatitis C Virus in Relation to Post-Transfusion Hepatitis

ALAN E. WILLIAMS, PH.D. and ROGER Y. DODD, PH.D.

Jerome H. Holland Laboratory
American Red Cross, Rockville, MD 20855

ABSTRACT

Natural history studies conducted over the past 15 years have shown that parenterally transmitted non-A non-B hepatitis infection frequently results in an indolent chronic disease with serious long-term consequences. The recent identification of nucleic acid sequences comprising the genome of hepatitis C virus (HCV) has allowed the development of a serological assay based upon recombinant viral proteins specifically associated with the major agent of non-A, non-B hepatitis infection. The HCV antibody assays have now been applied to sera from blood donors worldwide, as well as various population samples with increased hepatitis risk in the course of clinical trials conducted in both Europe and the United States. Data from these studies provide further encouragement that assays based on the hepatitis C virus recombinant proteins are highly specific for the major agent of non-A non-B hepatitis and will provide a firm basis for blood donor screening and future diagnostic tests.

Introduction

A recent editorial in the Lancet entitled: "Will the real Hepatitis C Stand Up?" accompanied several of the earliest published reports of the worldwide sero-epidemiology of HCV, and sought to convince the reader that after 15 years of relative frustration, the major agent of non-A, non-B hepatitis had been specifically identified. The overview of post-transfusion non-A, non-B hepatitis presented here will outline some of the keynotes in the history of this field, as well as present some of the early European and U.S. clinical trial data which led to the editorial's optimism that a new era in the understanding of human liver disease has now begun.

Non-A, Non-B Hepatitis Description

With the advent of specific serological testing for the agents of hepatitis B and hepatitis A, the existence of an unrecognized agent of viral hepatitis became evident. Since 1975, the diagnosis of this uncharacterized form of liver disease has been largely exclusionary, and the infection appropriately became known as non-A, non-B hepatitis (also referred to as NANBH). Owing to the non-specific
nature of non-A non-B hepatitis diagnostic criteria, it is likely that at least some of the cases of observed biochemical manifestations of liver damage are a result of hepatocyte exposure to injuries other than viral agents. Known causes of short- and long-term elevations of the liver transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood serum include: autoimmune disease, alcohol ingestion, exercise, obesity, and poorly defined non-viral hepatocyte injury associated with the receipt of homologous blood components (table I).

While the primary non-A, non-B agent of concern in the western world is the virus associated with parental exposures inherent in blood transfusion, IV drug use, and needle-stick injuries, an enteric water-borne form of non-A non-B hepatitis has been reported from India and other less-developed countries for which a calicivirus-like agent has now been identified through serologic and electron microscopic studies. With recent advances in knowledge regarding the specific agents of viral hepatitis, the previous designations of HAV for hepatitis A and HBV for hepatitis B have now been joined by the designation HCV for the parenterally transmitted form of non-A non-B; HDV for the ribonucleic acid (RNA) satellite virus associated with hepatitis B infection (known also as the delta agent); and HEV for the enteric form of non-A non-B infection (table I). It is not currently clear whether or not other unknown agents have an important role in the etiology of human viral hepatitis.

While the existence of a non-A non-B form of hepatitis has been known since 1974, it has only been with recent data from prospective studies of post-transfusion hepatitis patients that the full spectrum of disease related to the non-A non-B agent has been fully recognized. Hepatitis B infection is known to result in chronic liver disease in approximately six to 10 percent of patients with acute HBV infections, following which approximately 50 percent of these patients progress to chronic active hepatitis with increased risk of subsequent liver cirrhosis and primary hepatocellular carcinoma (PHC). The clinical outcome of non-A, non-B hepatitis infection, previously thought to be largely uneventful, has now been linked with an even greater tendency to produce chronic liver damage than hepatitis B, with persistent ALT elevations developing from more than 50 percent of acute non-A, non-B cases. Once chronic non-A, non-B hepatic disease is present, it appears that as many as one half of such patients progress to an indolent form of chronic active hepatitis with an increased risk of subsequent liver cirrhosis and PHC.7

Beginning with the first definition of non-A, non-B hepatitis in 1974, an intense flurry of activity surrounded the search for a candidate viral agent. Despite knowledge of many of the epidemiologic characteristics of non-A non-B hepatitis and an intensive search for a specific immunologic or virologic marker with which to define this infection, success has ultimately required 15 years of epidemiologic and clinical observation, replete with numerous descriptions.
of unproven candidate viral particles and serological markers in the medical literature.²

The first milestone in the characterization of non-A, non-B hepatitis infection occurred in 1984 with the transmission of infection and asymptomatic viral hepatitis to non-human primates.⁸ Even with the availability of this animal model, electron microscopic and serological attempts to find a specific disease agent were unfruitful.

**Surrogate Testing of Donated Blood**

In 1986, the lack of success in identifying a specific marker for non-A, non-B hepatitis infection led the blood supply complex to adopt surrogate methods of donor screening to reduce the incidence of transfusion-associated hepatitis, estimated at that time to be approximately 10 percent. These methods included routine assay of liver transaminase levels in donated blood, as well as testing for antibody to hepatitis B core antigen (anti-HBc). Because these two surrogate markers appeared to define essentially different donor populations, each with an increased risk of transmitting the non-A, non-B agent, it has been estimated that the resulting effect was a 50 percent drop in the incidence of post-transfusion hepatitis, at a cost of approximately 5 percent of the existing donor pool.²¹ The introduction of AIDS risk self-deferral measures and HIV-1 antibody screening of blood donors in early 1985 also served to reduce further the pool of donors capable of transmitting non-A non-B hepatitis infection, so that the incidence of post-transfusion infection is now estimated to be under two percent.

**HCV: Discovery and Characterization**

Over the past three years, several remarkable advances have been made in defining the specific agent of non-A non-B hepatitis, now called hepatitis C virus (HCV). The characterization of proteins and antibodies specifically associated with hepatitis C infection began at the Chiron Corporation in 1985, with attempts to clone blindly and to express fragments of nucleic acid from research materials containing the non-A, non-B agent without prior visualization or purification (table II). This process began with the high speed pelleting of known infectious plasma obtained from chimpanzees. The pelleted material was denatured and reverse transcribed to create complementary deoxyribonucleic acid (cDNA) sequences homologous to the total pool of native RNA and DNA sequences present in the plasma pellet. This cDNA was then inserted into plasmid vectors which subsequently expressed specific protein products by growth in *Escherichia coli*. While this technique resulted in the production of many protein-producing clones, selection of a viral protein specifically associated with non-A, non-B hepatitis remained a critical step in the process. This selection was accomplished by the testing of approximately six million individual clones by protein immunoblot

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant Hepatitis C Virus Protein Production</td>
</tr>
<tr>
<td>Viral RNA extracted from Hepatitis C virus infected primate plasma</td>
</tr>
<tr>
<td>Reverse transcription to cDNA</td>
</tr>
<tr>
<td>Cloned into expression vector (lambda gt11)</td>
</tr>
<tr>
<td>Clone identified expressing a viral polypeptide immune-reactive with plasma component from Hepatitis C virus chronic carrier (approximately 10⁶ clones screened)</td>
</tr>
<tr>
<td>Open reading frame expressed as fusion polypeptide in yeast culture</td>
</tr>
<tr>
<td>Fusion protein from three related clones (C-100-3) used as an antigen source in Hepatitis C virus antibody immunoassay</td>
</tr>
</tbody>
</table>
against a panel of sera derived from chronically infected and convalescent non-A, non-B patients.

One of several reactive clones (Clone 5-1-1) was identified through its specific reaction with the serum of a known HCV chronic carrier, and its absence of reaction with a battery of control sera. Upon further analysis, the nucleotide sequence contained in this clone corresponded to a viral RNA open reading frame (ORF) which, when subsequently expressed as a single fusion polypeptide in yeast cultures, formed the basis of a serologic screening test for antibodies specifically related to the etiologic agent of non-A non-B hepatitis. Following further work, this specific sub-genomic fragment of HCV RNA was used to develop overlapping cDNA sequences, ultimately leading to elucidation of the complete virus nucleic acid sequence. While HCV has not yet been visualized by electron microscopy, prior work conducted in chimpanzees, as well as the latest molecular virologic studies, have identified several properties of the elusive virus (table III).

Previous filtration studies in animals had characterized the non-A, non-B agent as a filterable virus with an approximate size of 30 to 60 nm, with a chloroform-sensitive lipid envelope. In vivo, the virus produces characteristic tubular-like structures in the cytoplasm of liver cells of experimentally infected chimpanzees. Additionally, in vivo titration studies, as well as the difficulty of EM visualization of the agent, imply a concentration of infectious virus in blood that is considerably lower than HBV.

Molecular biologic studies of HCV conducted over the past three years have rapidly expanded the list of known characteristics of the major non-A, non-B agent. The genome has now been shown to be a single positive-stranded linear RNA of approximately 10,000 nucleotides with a single large open reading frame. The nucleic acid structure of HCV, as well as its physicochemical properties, points towards its characterization as a possible member of the flavivirus family. This potential relationship of HCV to this large group of human RNA viruses, is further supported by the existence of minor sequence homology between the HCV genome and those of other known flaviviruses. This relationship has been further reinforced by a striking similarity observed between the pattern of hydrophilic and hydrophobic domains in the single polyprotein coded by the large ORF of HCV and well-known flaviviruses.*

HCV Seroepidemiology

The HCV Clone C-100-3 (an expanded version of the original clone C-100) produces a fusion protein containing 363

<table>
<thead>
<tr>
<th>TABLE IV Anti-Hepatitis C Virus Reactivity of National Institutes of Health Non-A Non-B Hepatitis Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Positive in 4/4 non-A, non-B chimps</td>
</tr>
<tr>
<td>2. Negative in 3/3 hepatitis B virus chimps</td>
</tr>
<tr>
<td>3. Negative in 4/4 hepatitis A virus chimps</td>
</tr>
<tr>
<td>4. Positive in 7/11 Hu non-A, non-B patients</td>
</tr>
<tr>
<td>5. Negative in 10/10 normal donors</td>
</tr>
</tbody>
</table>

* Houghton, M.: Personal communication.
amino acids from the non-structural portion of the HCV genome. This protein forms the basis of the screening and diagnostic serological test recently developed by Chiron Corporation in conjunction with Ortho Diagnostics. A radioimmunoassay based upon this cloned protein, when used in an assessment of the coded panel of well-characterized non-A, non-B sera assembled at the National Institutes of Health, was the first assay capable of reproducibly distinguishing sera known to be associated with non-A, non-B hepatitis infection. As shown in table IV, the HCV assay was reactive in four of four chimpanzees with known non-A, non-B infection, non-reactive in serum from three hepatitis B-infected and four hepatitis A-infected chimpanzees, and reactive in seven of 11 human non-A, non-B hepatitis patients. Ten pedigreed donor controls were non-reactive.

These early indications of a successful relationship between the hepatitis C serologic test and the clinical disease led to the implementation of comprehensive serological tests in the United States and around the world, both to develop further the epidemiology of this agent and to serve as clinical trial data to support diagnostic and blood screening licensure applications to the United States and European regulatory agencies. Of approximately 30,000 blood donors tested worldwide during clinical trials, the range of reactivity ran from a high of six percent in African donors to a low of 0.24 percent of donors in Finland (figure 1). Blood donors in the United States currently exhibit approximately 0.6 percent reactivity in this assay. Serologic testing was also performed on a broad spectrum of donors with known surrogate serologic results and patients known to have increased risk of non-A, non-B hepatitis infection. A summary of data from U.S. and European blood donors indicates that in those with only anti-HBc antibody (n = 436), or only ALT elevations (n = 777), hepatitis C antibody was present with a prevalence of 5.3 percent and 2.5 percent respectively. When both ALT and anti-core were present (n = 60), however, the rate of anti-HCV reactivity rose to 37 percent (figure 2).

Seroprevalence rates for U.S. blood donors with other markers, as well as other at-risk population samples in the U.S., are shown in figure 3. While the proportions of U.S. donors with surrogate markers found to be anti-HCV positive are somewhat higher than the combined U.S. and European data, verification of a meaningful difference between the U.S. and European data must await the testing of larger sample
sizes. Anti-HCV seroprevalence for various patient categories includes 60 percent in U.S. hemophiliacs, 15 percent in thalassemic patients, 20 percent in renal dialysis patients, a strikingly high rate of infection in intravenous drug abusers (76 percent), and an equally striking low rate of reactivity in gay men at 4.4 percent (figure 3). While the rates of anti-HCV in the first three patient groups roughly parallel the previous clinical experience of non-A, non-B hepatitis risk in these categories, the unusually high rates in IV drug users and unusually low rates in gay men may provide useful clues into the epidemiology of this agent. These may possibly reflect the ability of the current HCV assay to detect antibodies associated with ongoing HCV disease more efficiently than neutralizing antibodies associated with immunity.

HCV: Clinical Correlations

The first comprehensive assessment of HCV as an agent of post-transfusion non-A, non-B hepatitis (PT-NANBH) was conducted in the Netherlands, where the new assay was applied to serial sera from nine cardiac surgery patients with documented NANBH and nine matched controls. Four of the nine NANBH patients seroconverted to anti-HCV compared with none of the transfused controls. Furthermore, in 7/9 NANBH cases anti-HCV was evident either in a recipient sample or in stored serum from at least one of the implicated donors.19

A question commonly asked in the blood community is whether or not the anti-HCV test will be able to replace the rather cumbersome and expensive surrogate assays for ALT and anti-HBc currently being conducted on all donated blood. The replacement of these surrogate assays appears to be unlikely, primarily owing to the relationship of anti-HCV antibody development to the time course of HCV infection and disease. A useful approximation of the dynamics of seroconversion has been provided by a
prospective study of 34 transfusion-associated hepatitis cases from Spain in which 13 percent of the patients had developed anti-HCV six to eight weeks following transfusion, another 32 percent during the 20 to 28 week period, and another 34 percent in the 29 to 52 week period, for an approximate total of 79 percent seropositivity associated with clinical infection. Application of the anti-HCV test to post-transfusion hepatitis patients in the U.S. (figure 4) studied prospectively shows the existence of specific antibody in approximately 81 percent of those individuals with chronic infection, 71 percent in those with an indeterminate duration of disease, and only 32 percent prevalence in patients in the acute phase of illness.

The delayed onset of anti-HCV antibody has also been demonstrated in studies of community-acquired infection where sexual transmission of the virus is thought to account for much infection. Serological studies of non-A, non-B hepatitis patients without a history of transfusion have shown a 73 percent seroprevalence in chronic hepatitis patients vs. 45 percent in those with acute illness (figure 5). While the hepatitis C virus antibody assay appears to be highly specific for the agent of non-A, non-B hepatitis, it is recognized that the test is somewhat deficient for the diagnosis of acute phase illness. Much of the current effort in this field is currently directed at improving the sensitivity characteristics of the assay. The most immediate goal for improving the performance characteristics of this assay is to use the newly-sequenced entire HCV genome for the development of additional HCV viral proteins which may broaden the spectrum of antibody detection to include early non-structural and structural viral proteins, as well as provide the basis for confirmatory procedures to verify the specificity of reaction in those individuals found to be seropositive. Only then will it be known whether or not HCV represents all non-A, non-B hepatitis infection or if a search will continue for a non-A, non-B, non-C agent.

Acknowledgments

Much of the information discussed in this review represents personal communications from individuals involved in research in this area, data from clinical trials, and information presented at the First International Meeting on Hepatitis C Virus, held in Rome, September 14 and 15, 1989. The assistance and contributions of all these individuals are gratefully acknowledged.

References


