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VIRAL HEPATITIS C, D AND E

Viral Hepatitis C, D and E

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Preface

Until recently, the discovery of an unknown pathogenic virus depended on 'classical' virological methods such as demonstrating the virus by electron microscopy or detecting viral proteins with specific antibodies. However, with the development of gene engineering techniques, the discovery of new viruses can now be achieved by strictly molecular methods.

Hepatitis C virus is the first example of a virus that was identified by these molecular cloning techniques. The study of viruses causing post-transfusion non-A, non-B hepatitis, the existence of which was discovered in the mid-1970s, did not progress rapidly until recently because of repeated failure to detect the virus antibodies. This was despite the successful transmission of non-A, non-B hepatitis to chimpanzees and its characterization as a small enveloped virus. The reason for the limited progress was probably the generally low titer of virus present in clinical materials, the failure to isolate the virus in cell culture and a relatively poor immune response to the agent. However, the breakthrough in research on non-A, non-B hepatitis came with the molecular cloning of cDNA representing the viral genome of the non-A, non-B hepatitis virus, now called hepatitis C virus (HCV). This was achieved by Dr. Michael Houghton in the United States and by Dr. Terukatsu Arima of Japan in independent studies. An assay for antibody to HCV (anti-HCV) was developed by Dr. Houghton's group at the Chiron Corporation and has been evaluated with the sera from patients with various liver diseases such as acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma and with sera from normal blood donors and donors implicated in the transmission of non-A, non-B hepatitis to blood recipients. The results suggest that the test will be important in detecting persistent infection with HCV. Thus, there appears to be no remaining doubt that HCV is the major causative agent of post-transfusion non-A, non-B hepatitis. It is also significant that a high prevalence of antibody has been found in patients with chronic liver disease, including hepatocellular carcinoma, in various parts of the world. This finding will stimulate a great deal of research on a possible etiologic association between chronic HCV infection and liver cancer.

The discovery of HCV, the establishment of assay systems, and the prospect for developing a hepatitis C vaccine have generated much optimism for the prevention of infection with this virus through interdiction of transmission to blood recipients and immunoprophylaxis of infection in the general population. Achievement of these goals would have a considerable impact on public health by preventing acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma associated with HCV infection. Thus there is optimism that the progress currently being made in the control of hepatitis B will be repeated with hepatitis C.

Similar progress has been made in the study of another non-A, non-B hepatitis virus: the enterically transmitted form of hepatitis that is prevalent in India,

Myanmar, Nepal, southwestern China, central Asia and northern and western Africa. This virus, also called hepatitis E virus (HEV), was first described in 1980 and was shown to be caused by a small enteric virus by experimental transmission to a volunteer. More recently, the virus has been experimentally transmitted to marmosets, macaques and chimpanzees at several research institutes around the world. The virus has been most readily demonstrated in the bile of experimentally infected animals. Virus derived from bile was used to molecularly clone cDNA derived from the viral genome by Dr. Gregory Reyes, of GeneLabs, Inc., in the United States. It is hoped that these studies will lead to a simple serologic test to replace immune electron microscopy and indirect immunofluorescence in detecting antibody to the virus. It is hoped that progress in the study of HEV will lead to rapid diagnosis of the disease and, eventually, vaccination and other forms of immunoprophylaxis. Control of this virus would have a major impact on public health in the regions of the world where it is endemic.

A third 'non-A, non-B' hepatitis virus was also discussed at this symposium. Hepatitis δ virus (hepatitis D virus, HDV) is a defective virus that requires coinfection with hepatitis B virus for its replication and it causes a more severe hepatitis when superimposed on either acute or chronic HBV infection. The agent, the genome of which was also reverse-transcribed, cloned and sequenced by Dr. Houghton, more closely resembles certain pathogens of plants (viroids, virusoids, satellite RNAs and satellite viruses) than any animal virus. Studies of the replication of HDV and similar agents have great implications for a better understanding of RNA processing. Since HDV requires infection with HBV, the successful application of immunoprophylaxis programs for HBV should eradicate HDV as well.

This proceeding is the record of an international symposium on non-A, non-B hepatitis which was the first to be held since the successful molecular cloning of the genomes of hepatitis C and E viruses. We, the organizers of this symposium, were very grateful to the many leading researchers in the study of viral hepatitis who were able to attend and participate in the presentation and discussions at this critical juncture in hepatitis research.

This symposium was one of a series of international symposia held to commemorate the 100th anniversary of the Nihon University. We believe that the proceedings of the symposium will be a most useful addition to researchers in this field.

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27 March 1990

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Section I

HEPATITIS C

Viral hepatitis C, D and E
T. Shikata, R.H. Purcell and T. Uchida, editors

CHAPTER 1

Non-A, non-B hepatitis. Success and failure in an era of molecular biology

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Clues and suggestions for at least three forms of viral hepatitis have existed for more than 20 years. Epidemiological patterns and multiple attacks in humans observed in the 1960s and 1970s were not clarified until specific serodiagnostic methods for hepatitis A (HAV) and B (HBV) were widely used. Non-B post-transfusion hepatitis (PTH) was clearly documented in 1974 and these infections were shown to be non-A in 1975 when serological immunoassays for HAV infection were applied. Since then we have seen steady progress in understanding the epidemiological, clinical, and biophysical features of the non-A, non-B (NANB) hepatitis agent, culminating in 1988 with the discovery of NANB virus through molecular cloning. Why did it take almost 20 years to discover and characterize this agent? The first AIDS virus (HIV-1) was discovered and characterized in less than five years after the disease was identified. Other infectious agents for humans and animals have followed rapidly after identification of the disease. In the following sections I have retrospectively analysed the NANB hepatitis journey—successes and disappointments—over a period of 18 years that culminated in 1988 with the discovery of the hepatitis C virus (HCV) through molecular cloning. The many years of adding pieces to the puzzle by many people provided the background for the last piece of the puzzle to fall into place.

Annual accomplishments

In Table 1, selected key accomplishments are identified with a typical published reference. Actual awareness of each milestone probably preceded the publication date by one or two years. Collectively, these studies gave hepatologists a clear picture of the epidemiology and serious clinical consequences of transfusion-associated NANB hepatitis. In addition, important biophysical characteristics of a blood-borne virus were well in hand by 1987. The ability to infect and diagnose disease in chimpanzees was key to many of these accomplishments. The following conclusions about NANB hepatitis were on sound ground: (1) high (10%) risk from injected blood or blood products; (2) frequent (20–70%) NANB in sporadic disease; (3) chronic symptomatic and asymptomatic viremia; (4) titres of 10^3–10^6 infection units/ml in chimpanzee

TABLE 1 Annual progress and milestones in the discovery of the transfusion-associated NANB hepatitis virus (HCV)

Year	Event	Reference
1970	Clues for three types of hepatitis	[1]
1971	HBV screening gave low detection	[2]
1972	Third generation HBV tests	[3]
1973	HAV discovered by IEM	[4]
1974	Non-B PTH identified	[5]
1975	50–80% PTH due to NANB	[6]
1976	Serious chronic disease	[7]
1977	Multiple hepatitis attacks in humans	[8]
1978	Chimpanzees infected with NANB	[9]
1979	Ultrastructural hepatocyte changes	[10]
1980	Water-borne NANB identified	[11]
1981	Surrogate ALT and anti-core markers	[12]
1982	20–70% sporadic hepatitis is NANB	[13]
1983	Two NANB PTH agents	[14]
1984	Associated with hepatocellular carcinoma	[15]
1985	Togavirus-like properties	[16]
1986	Epidemic NANB-like PTH in chimps	[17]
1987	Delta virus cloned	[18]
1988	HCV cloned	[19]

blood; (5) serious long-term chronic hepatitis; (6) risk of hepatocellular carcinoma; (7) HBV-like epidemiology; (8) small, $CHCl_3$-sensitive virus.

Disappointments

Beginning in 1978, published and patent literature have suggested that practical solutions to NANB hepatitis are at hand. Some 20–30 reports of specific antigen-antibody systems have appeared [20]. Two or more serologically distinct agents have been reported [21]. Virus-like particles visualized by electron microscopy have been reported frequently, but not reproducibly confirmed [22]. Reports that NANB may be a serologically silent HBV variant [23] or a retrovirus infection [24] have been confusing. Attempted isolations in tissue culture have failed [25]. Although the above studies are here classed as disappointments in our direct discovery of the NANB hepatitis virus, the knowledge gained from 'negative' results often contribute equally to guiding studies toward 'positive' findings.

Success

Houghton and colleagues [19] persevered for several years in identifying HCV by cloning and identifying the viral genome through expression screening. The three

strategic requirements for successful isolation of a rare virus nucleic acid by immunoscreening are: source of titred virus, source of qualified antiserum, and verified molecular biology skills. After acquiring blood plasma with a high virus titre, the major risk in these three requirements was the commitment that the serum from a transfusion-associated hepatitis patient with well diagnosed chronic hepatitis will have antibodies to a dominant viral antigen epitope. With an antiserum in hand, success was then assured based on the statistics of finding one NANB transformant in a library of 1 to 10 million other transformed cells—a task well-suited for modern molecular biology.

The people and research groups that built the foundation of knowledge that permitted successful discovery of HCV via molecular genetics over almost two decades were not rewarded in the major final breakthrough. Much remains to be done before benefits arrive at the NANB patient level. The way is now open, and the journey is the reward.

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