Discovery of Hepatitis C Virus*

2020 Nobel Prize in Physiology or Medicine

Aunji Pradhan, Sachin Kumar Tripathi and Saumitra Das

The 2020 Nobel Prize in Physiology or Medicine was awarded jointly to Harvey J. Alter of the National Institute of Health (NIH), Michael Houghton of the University of Alberta and, Charles M. Rice of Rockefeller University. The Nobel Assembly at Karolinska Institute has awarded them the Nobel Prize for the discovery of Hepatitis C virus (HCV).

Viral Hepatitis

Hepatitis refers to the inflammation of the liver, which might progress to cirrhosis and, in some extreme cases, to hepatocellular carcinoma. Hepatitis virus is the most common cause of hepatitis along with some non-viral factors such as alcohol consumption, auto-immune disease, and drugs. Five different types of RNA/DNA viruses have been reported to cause hepatitis worldwide. They contribute substantially to the global burden of hepatic diseases [1]. According to the latest *WHO Global Hepatitis Report*, hepatitis A virus (HAV) infection caused 114 million cases of acute hepatitis in 2015, while 257 million people were living with chronic hepatitis B virus (HBV) infection and 72 million with chronic hepatitis C virus (HCV) infection that year. Due to their capacity to establish chronic infections, HBV and HCV are the two major causes of morbidity and mortality worldwide with 1.34 million deaths reported in 2015, a 63% increase from 1990, mainly due to HCV infection [1]. ‘

In 1940s, the first infectious viral agent responsible for hepatitis was reported to be the hepatitis A virus. It is an RNA virus

Aunji Pradhan is a PhD scholar in the laboratory of Prof. Saumitra Das, at IISc. She has been working on the role of exosomes in HCV induced pathogenesis.

Sachin Kumar Tripathi is also a PhD scholar in the laboratory of Prof. Saumitra Das at IISc. He has been working on the role of lncRNA in HCV pathogenesis.

Saumitra Das is a Professor of Molecular Virology at the Department of Microbiology and Cell Biology, IISc. Currently he is the Director of National Institute of Biomedical Genomics. His laboratory at IISc is working on various aspects of HCV.

*Vol.25, No.12, DOI: https://doi.org/10.1007/s12045-020-1089-5

belonging to the *Picornaviridae* family, which is transmitted via polluted water or food and causes mild to severe illness. Similarly, a DNA virus, known as the hepatitis B virus belonging to the *Hepadna* family is another major cause of hepatitis. It is transmitted through blood and body fluids and has a long incubation period. It causes serious disease manifestations like cirrhosis and liver cancer [2]. For the discovery of this hepatitis B virus, Baruch Blumberg was awarded the 1976 Nobel Prize in Physiology or Medicine [3]. The discovery of these viruses has been major milestones that have revolutionized medicine and substantially improved human health. The discovery of HAV and HBV has aided the development of effective diagnostic tests and vaccines, and has significantly reduced the risk of transmission of hepatitis infection via blood transfusion worldwide.

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Discovery of non-A, non-B Hepatitis

Even though the discovery of hepatitis A and hepatitis B virus significantly enhanced the detection of infectious agents, and reduced the cases of blood-borne hepatitis, Harvey Alter and his group still observed significant cases (10%) of persistent hepatitis infection and liver cirrhosis in transfusion patients [4]. This observation led them to consider different infectious agents unrelated to HAV and HBV in the blood of donors, which they referred to as non-A, non-B hepatitis (NANBH) [5, 6]. This new form of hepatitis with milder symptoms and a longer incubation period became increasingly prevalent. It was also easily transmissible to chimpanzees (*Pan troglodytes*) [7–9].

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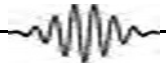
Identification of Hepatitis C Virus

Identification of non-A, non-B hepatitis has been a major challenge for over a decade due to the lack of standard serological assays and low yield of the virus. In 1989, Michael Houghton and his group took the approach of generating a DNA library from infected blood for the identification of the novel antigens of the new virus causing hepatitis. Firstly, they generated a ran-

dom primed complementary DNA library in bacteriophage λgt11 from plasma containing uncharacterized non-A, non-B hepatitis, which was further screened for rare clones expressing viral antigen with serum from the patient diagnosed with NANBH as well as from the uninfected controls or patients with type A or type B hepatitis. After screening nearly 106 clones, the research group finally found one positive cDNA clone (5-1-1) that reacted with sera from persons with NANBH, but not with sera from uninfected controls or patients with type A or type B hepatitis. Further, they went on to characterize the nature of the antigen using the hybridization assay to determine the genomic nature of the antigen, i.e., either its DNA or RNA. They observed that cDNA clone hybridized specifically to the total RNA extracted from the infectious chimpanzee liver but not to the total DNA as well as not to total RNA derived from control, uninfected chimpanzee livers. This observation helped them conclude that the antigen was derived from an exogenous RNA molecule, and it was single-stranded in nature. The size of the RNA was analyzed using the northern blot assay. In this method of analysis, RNA molecules from the infected tissue were separated on a denaturing formaldehyde agarose gel by electrophoresis, transferred to nitrocellulose membrane followed by hybridization with a probe. This led them to conclude that the approximate size of the RNA molecule was to be between 5,000 to 10,000 nucleotides. A strong hybridization signal was obtained for a population of RNA molecules that bound to oligo(dT)-cellulose indicating the presence of either a 3′ poly(A) sequence or an A-rich tract in the molecule. Furthermore, the cDNA strand that hybridized with plasma-derived RNA was found to be complementary to the strand encoding the 5-1-1 open reading frame (ORF), indicating that this RNA is positive-stranded with respect to translation of this viral antigen. They termed this newly characterized non-A, non-B hepatitis virus as hepatitis C virus (HCV) related to the *Togaviridae* or *Flaviviridae* family [10].

Hybridization assays led Michael Houghton's group to conclude that the hepatitis antigen was derived from an exogenous RNA molecule, and it was single-stranded in nature. The size of the RNA was analyzed using the northern blot assay, leading them to conclude that the approximate size of the RNA molecule was to be between 5,000 to 10,000 nucleotides.



Development of an Immune Assay for the Detection of Circulating Antibodies for Hepatitis C Virus

After the identification and isolation of the cDNA clone of non-A, non-B hepatitis, designated as hepatitis C virus, Michael Houghton's group developed the recombinant antigen-based assay for the detection of HCV antibodies in the human blood/serum.

After the identification and isolation of the cDNA clone of non-A, non-B hepatitis, designated as hepatitis C virus, Michael Houghton's group developed the recombinant antigen-based assay for the detection of HCV antibodies in the human blood/serum. The group constructed continuous ORFs that expressed the viral polypeptides, which act as an antigen. The ORFs were then expressed in yeast in fusion with human superoxide dismutase (SOD) that facilitates the efficient expression of the protein in yeast. The SOD/HCV polypeptide, referred to as C100-3, containing 363 viral amino acids, was produced in high levels in recombinant yeast. The protein was further purified and used to coat the wells of the microtiter plate so that it can identify, capture, and measure the circulating HCV antibodies. Detection of the bound antibody was achieved with a radioactive secondary antibody. This recombinant based assay has been one of the greatest developments in the detection of HCV antibodies in the non-A, non-B hepatitis serum of the patients and blood donors to reduce the risk of blood transmitted hepatitis. This method ascertained that HCV was the major cause of NANBH throughout the world and allowed for rapid screening of donors for HCV along with HAB and HBV to prevent transfusion-associated hepatitis [11].

Establishment of an Infectious Cell Culture Model System

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Can HCV alone cause hepatitis? To answer this question, Charles M. Rice at Washington University, St. Louis, and other groups working on RNA viruses started studying uncharacterized regions at the end of the HCV genome. They cloned the virus, which could replicate and cause hepatitis in chimpanzees. Highly variable viral quasi-species and less viral load were the main hurdles in cloning the virus. They used the serum obtained from a patient in the early phase of infection (designated as H77). They isolated the virus, reverse transcribed, and subcloned it into a plasmid containing 5′ and 3′ terminal HCV consensus sequences. They

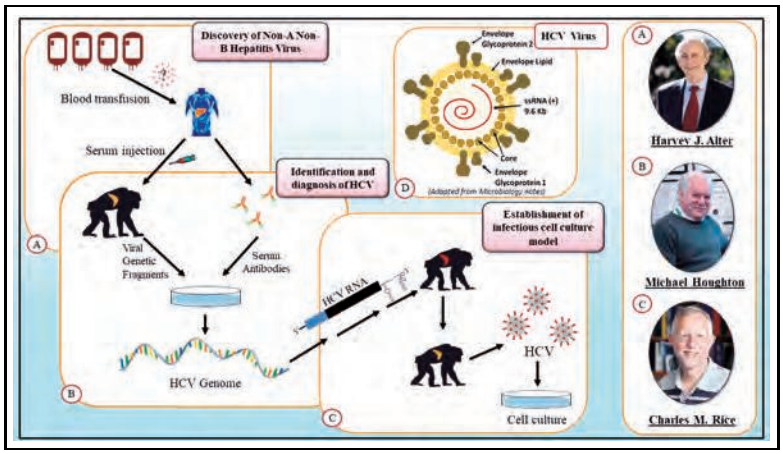

Figure 1. Nobel Prize 2020 in Physiology or Medicine: **(A)** Harvey J. Alter observed and investigated hepatitis in patients who had received blood transfusions from non-A, non-B blood donors. Blood samples from these patients could transmit the disease to chimpanzees as well. They named it as non-A, non-B hepatitis (NANBH). **(B)** Michael Houghton isolated and characterized the cDNA clone from the genetic fragments of the new virus using infected chimpanzee's blood. Later, along with H J Alter, he developed a recombinant antigen based diagnostic test for HCV. **(C)** Charles M. Rice created an engineered version of the hepatitis C virus and showed it was sufficient to cause hepatitis in chimpanzees. This proved that hepatitis C virus was one of the major causes of hepatitis during blood transfusions. **(D** Diagrammatic representation of hepatitis C virus (HCV). Image credit: https://www.nobelprize.org/

screened several clones and injected them into a chimpanzee. They could not detect circulating HCV RNA, increased liver enzymes, or histopathology, which typically indicate hepatitis [12]. Based on these results and sequencing, Prof. Rice concluded that genetic variations in the HCV genome at the 5′, envelope protein region, and at the 3′ region could hamper virus replication [13]. Scientists then used genetic engineering to develop HCV RNA to remove certain genetic variations. When this RNA was injected into a chimpanzee, not only they were able to detect the virus, they also observed changes in liver enzymes, consistent with virus replication in the liver. They did additional experiments to prove that the virus alone could cause hepatitis [12]. Their investigation also identified the functional component of HCV genomic RNA. These clones helped study virus replication, evolution, pathogenesis, and host immune response. Later, Prof. Rice with collaborators made other clones for replication studies. He also made virus permissive liver cancer cell lines for the study of HCV infection and identified mutations in the HCV genome that helped the virus adapt to growth in the laboratory. He worked on the receptor (CD81, scavenger receptor B1, claudin-1, and occludin) responsible for HCV entry, as well as made a mouse model to study HCV biology/pathogenesis [14–17]. These studies helped to understand the viral life cycle, and aided research on antivirals and vaccine development.

Implication of the Nobel Winning Discovery

The discovery of hepatitis C virus is considered a landmark in identifying the major causes of viral hepatitis. This discovery allowed the isolation of the HCV clone and the development of a highly sensitive assay to detect the presence of HCV antibodies.

The discovery of hepatitis C virus is considered a landmark in identifying the major causes of viral hepatitis. This discovery allowed the isolation of the HCV clone and the development of a highly sensitive assay to detect the presence of HCV antibodies. These rapid tests have eliminated the risk of transmission of hepatitis via blood transfusions, leading to the prevention of transfusion-mediated spread of hepatitis worldwide. Further, the establishment of the infectious cell culture system by Rice has laid the ground for research on the viral lifecycle inside the human host and its pathogenesis, which in turn has helped in identifying various molecules that can be used as potential antiviral drugs candidates to treat hepatitis. Their discovery has also immensely improved our understanding of the antiviral immune response of the host, which will help in the development of HCV vaccine in near future.

Concluding Remarks

The incredible observations and research work of Alter, Houghton, and Rice, and their research groups have led to the discovery of hepatitis C virus, a non-A, non-B hepatitis virus (*Figure* 1). Their findings have helped eliminate the risk of transmission of hepatitis C via blood transfusion, as well as aided the development of effective drugs for its cure. As a result, HCV induced hepatitis is now considered a curable disease with 95% recovery upon antiviral treatment. This breakthrough achievement has already benefitted millions of individuals worldwide, and has provided a strong base for further research on generating vaccines against HCV as well as other viruses.

Acknowledgment

Saumitra Das acknowledges the Centre of Excellence for Hepatitis C Virus Research (HCV-COE), Department of Biotechnology, and J. C Bose National Fellowship from SERB. Sachin Kumar

Tripathi is supported by a CSIR Fellowship.

Suggested Reading

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Address for Correspondence
Aunji Pradhan
Sachin Kumar Tripathi
Department of Microbiology
and Cell Biology
Indian Institute of Science
Bengaluru 560 012
India.

Saumitra Das
National Institute of Biomedical
Genomics
PO: NSS
Kalyani 741 251, India
Email: sdas@iisc.ac.in