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31 Years of Discovery and the Progress of Hepatitis C Virus: 2020, Nobel Prize in Physiology or Medicine

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Abstract

Nobel Prize, 2020 in Physiology or medicine were jointly given to two American scientists Harvey J. Alter and Charles M. Rice and one British Scientist Michael Houghton for the discovery of Hepatitis C virus. The Nobel committee considered four decades of research on discovery and study of this virus. Prof Alter first identified the causative agent of transfusion associated unknown hepatitis, Prof. Houghton discovered and named this causative agent Hepatitis C virus and Prof. Rice developed *in-vitro* adaptable strains of this virus and discovered host cellular receptors for its entry.

Introduction

The Nobel Prize in Physiology or Medicine 2020 was awarded to three world famous virologists for their phenomenal research on a liver borne virus, Hepatitis C (HCV). They were awarded due to their contribution in discovery and unravelling the basic HCV biology and aetiology to fight against a global health problem that already affects more than 70 million people worldwide. The disease has 1 - 1.9% prevalence in India and causes liver inflammation which may develop fibrosis, cirrhosis, Hepatocellular Carcinoma (HCC) and untimely death [1,2]. In 2016, WHO estimated that approximately 40,000 people have died from HCV infection worldwide, mostly due to cirrhosis and HCC [1]. However, the death rate is gradually decreasing with more HCV research, proper screening of the patients, blood samples and development of potent, pangenotypic therapeutics since the discovery of this RNA virus in 1989 [3].

On 5th October 2020, Monday, The Nobel Assembly at Karolinska Institutet announced the names of the two Americans, Harvey J. Alter and Charles M. Rice, and a British Scientist Michael Houghton for the Nobel Prize, 2020 in Medicine or Physiology for the discovery of the hepatitis C virus. Announcing the prize in Stockholm, Sweden, the Nobel Committee noted that the trio's work unravelled a major source of blood-borne hepatitis that couldn't be explained by the hepatitis A (HAV) and B (HBV) virus and saved millions of lives (https://www.nobelprize.org/prizes/medicine/2020/press-release/). The Nobel committee also briefly described about other two types of major hepatitis viruses like HAV and HBV.

Around 1940, two main types of infectious substances responsible for liver inflammation or hepatitis had been de-

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scribed. In 1973, HAV was first discovered from fecal samples collected from infected human volunteers as visualised by immune electron microscopy [4]. This virus generally transmitted by polluted water or food causes acute but little long term impact on affected individuals. The second type, HBV, transmitted through blood or bodily fluids represented a much more serious threat or chronic condition which leads to development of cirrhosis and liver cancer. This virus was discovered in 1968 by the American physician Baruch Blumberg for which he was awarded the 1976 Nobel Prize in Physiology or Medicine [5].

Harvey J. Alter: The mysterious illness of post blood transfusion patients could be called as "Non A, Non B Hepatitis".

In late 1960s Harvey J. Alter, a clinician in National Institutes of Health, USA (NIH) blood bank worked on occurrence of transfusion related hepatitis in patients who had received blood transfusions from the NIH blood bank. Although all the blood samples were tested for newly developed screening method for HBV before transfusion, unfortunately a large number of hepatitis cases prevailed. Tests for HAV infection were also available this time, and it became evident that HAV was not responsible for these unexplained cases. In 1975, Alter and his group first demonstrated this unexplained cases as Non A, Non B hepatitis (NANBH) where finding of serological tests for HAV and HBV showed most of the cases of transfusion associated hepatitis did not have serological markers of either of these two viruses [6,7]. Interestingly they noticed this NANBH infection was sometimes independent of blood transfusion and transmission was shown to occur sporadically within a community [8].

To solve this mystery more investigations were needed and Professor Alter and his group decided to delve further and develop a potent animal model for this viral infection. Alter and his group used plasma or serum from four acute or chronic NANBH mediated Post Transfusion Hepatitis (PTH) patients and a symptomless blood-donor (her blood was given to two patients in whom hepatitis developed later), inoculated intravenously into five healthy chimpanzees at the Laboratory for Experimental Medicine and Surgery in Primates (L.E.M.S.I.P) in Sterling Forest, New York. The group observed that hepatitis was detected by biochemical and histological analysis in all five chimpanzees at a mean incubation period of 13.4 weeks without any serological evidence of HAV and HBV. Control (uninoculated) did not show any hepatic infection by both biochemical and histological detection. Professor Alter published these results in The Lancet on 4th March 1978 and suggested chimpanzee could be an animal model for NANBH [9] (Figure 1). Other groups reported that there could be possible existence of multiple NANBH agents or it could be a variant form of HBV in human [10]. Additionally, one NANBH agent was shown to exhibit large membranous cytoplasmic tubules within the liver cells of infected chimpanzees 10 and thus it was termed the Tubule Forming Agent (TFA) [11]. The TFA was shown to be filterable through 80nm filters and it was suggested that this may be related with a viral agent, probably from Gaviridae, Flaviviridae families or uncharacterized new virus [12].

Despite this progress, further information on aetiology and isolation of the viral strain (s) responsible for this unknown post transfusion hepatitis remained obscure for over a decade. Many groups did try to investigate but failed to find the antigen or antibody, nucleic acids or any detection methods for the patients associated with this NANBH specific agent(s) [13].

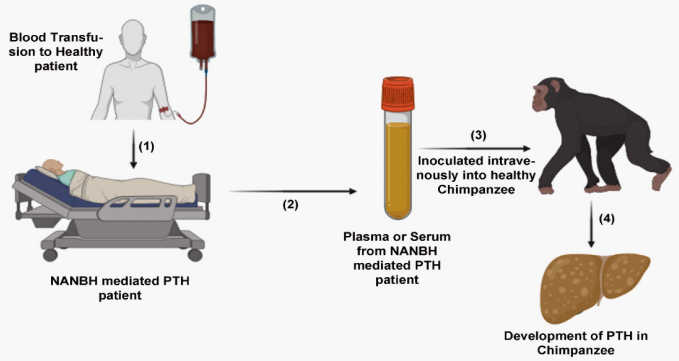

Figure 1: Schematic representation of NANBH mediated infection in Chimpanzee from NANBH infected human blood plasma or serum. Where NANBH refers to Non A Non B hepatitis and PTH refers to post transfusion hepatitis.

Michael Houghton: The causative agent of 'Non A and Non B hepatitis' is nothing but HCV.

Michael Houghton from Chiron Corporation (now part of Novartis), in a parallel investigation by one of the co-investigator of his laboratory Kang-Sheng Wang, was successful in creating fine-mapping B cell epitopes of the delta antigen using a λgt11 expression cDNA library derived from infectious Hepatitis D virus plasma. The historical success of this approach provided a strong encouragement to Prof. Houghton to apply this approach to isolate the causative agent(s) from infectious NANBH chimpanzee 910 plasma (collected from Chimpanzee #910, which had titre rate 10^6 CID/ml) produced by Dan Bradley [14]. He prepared a λgt11 expression cDNA library for NANBH similar to Wang but failed to isolate true NANBH clones from the total RNA collected from Bradley's plasma samples after screening with convalescent individual's sera of NANBH patients [15]. Keeping this result in his mind Prof. Houghton and his group tried again to generate another c-DNA library derived from same Chimpanzee 910 plasma, but this time they considered total nucleic acids samples (both DNA and RNA) instead of only total RNA. Furthermore, they decided to screen those cDNAs from individuals diagnosed with chronic hepatitis (NANBH) infection presenting unusually high serum alanine amino transferase elevations, a marker of severe liver damage.

Each individual clone in λgt11 expression vector with patient derived serum samples were screened and only few positive clones were identified. Qui-Lim Choo, a researcher in his laboratory, labelled one of the positive clones as 5-1-1. Southern Blot analysis indicated that 5-1-1 was neither derived from chimpanzee nor human genome. Instead, 5-1-1 and subsequent overlapping clones were derived from a library hybridised to a large single stranded RNA molecule with approximately 9,600 nucleotides that was present only in infected samples (NANBH). Sequencing results indicated that the nucleotide sequence in this foreign RNA molecule had a close homology with Flaviviral genome. Prof. Houghton named this virus as HCV and for the first time he presented the molecular identification of HCV in a public seminar organised by University of San Francisco in 1988 and it was published in Science on 21st April, 1989 [3] (Figure 2).

Subsequently, after this investigation, Prof. Houghton and his group used 5-1-1 clone to produce c100-3 antigen in recombinant yeast and circulating antibodies to c100-3 were developed and checked by enzyme immune assay [16]. This discovery opened a new door for the development of first generation blood screening test for HCV antigen in most blood banks worldwide.

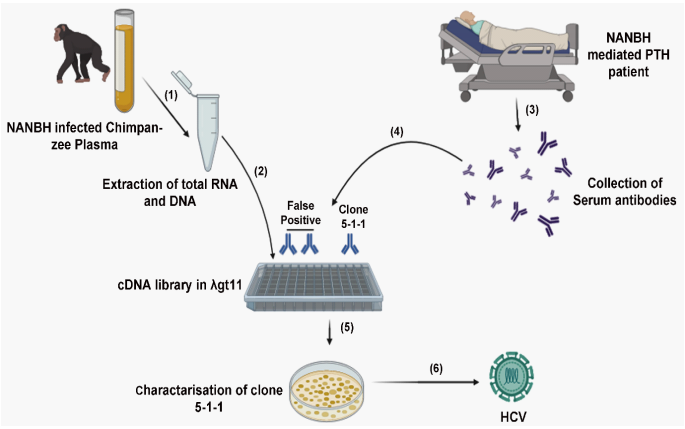

Figure 2: Schematic representation of molecular isolation and identification of HCV.

Charles M. Rice: Laboratory made molecular clone of a single HCV strain causes pathogenicity by itself.

In late 90s, even after several years of its discovery, Hepatitis C still was taking a huge toll worldwide. To develop useful therapeutics and potent vaccines, in vitro investigations in both animal models and cell culture system was urgently needed to unravel its diversity and life cycle. Charles M. Rice, a molecular virologist, working at Washington University, USA was exploring Sindbis and Yellow Fever viruses and produced infectious Flavivirus RNA in his laboratory. Shortly thereafter his publication in 1989 [17], Rice received a phone call from Stephen Feinstone, a researcher at the US Food and Drug Administration, suggesting Rice modifies his yellow fever vaccine strain to produce a vaccine for HCV. Rice accepted this challenging work and switched to HCV research from yellow fever.

In 1997, Professor Rice first produced a clone of infectious HCV that could be used to infect liver cells of chimpanzees only. This was an interesting finding that indicated for the first time that HCV particle could be produced within chimpanzee and moreover, revealed the type of cell lines the virus would allow for their entry and replication in a laboratory [18,19] (Figure 3A).

At the same time virologist Volker Lohmann developed a HCV subgenomic replicon system that allowed HCV to replicate in liver cancer cell lines [20]. Unfortunately the rate of replication of that particular HCV RNA in that liver cancer cell line was very low and was difficult to process for molecular studies.

Rice devised to develop a more permissive liver cancer cell line and worked on to identify the mutation in HCV that actually helped the virus to grow in *in- vitro* conditions. In 2005, Japanese molecular virologist Takaji Wakita and German virologist Ralf Bartenschlager and Rice from The Rockefeller University, established a new ground of HCV research to develop a HCV RNA, isolated from a Japanese patient fulminant hepatitis that could be forced to replicate in hepatic cell lines grown in laboratory dishes and produced filterable infectious HCV particles [21,22]. As a whole, this finding allowed researchers to investigate for the first time the entire lifecycle of HCV in their laboratory conditions (Figure 3B).

Being a virologist at The Rockefeller University, Rice has been involved in many research projects related to entry, replication and HCV assembly. Among those findings, one of the key finding was identification of a major receptor for HCV entry, Claudin-1(CLDN-1) which is found at tight junctions between epithelial tissues in liver. Rice along with his post-doctoral researchers observed that lentiviral HCV pseudoparticles (HCVpp) failed to infect livers cell when this receptor was not present in host cell membrane [23]. Joined with another important previously discovered host membrane receptor CD81 [24] and SR-B1 [25], discovery of CLDN-1 made an indispensable trio for HCV entry into hepatocytes (Figure 3C).

But the story behind the HCV entry was not complete with the discovery of this trio receptor, more research was needed to develop an effective animal model for HCV. In a 2009 Nature article, Prof. Rice and his two post doctorate fellows Alexander Ploss and Matthew Evans described the last major receptor protein Occludin-1 (OCLN-1), that HCV needs to enter mouse as well as human cells,. With the discovery of this receptor Prof Rice found the missing factor required for viral entry in human and mouse [26] (Figure 3C).

After successful discovery of cellular receptors and development of cell culture adaptable strain for HCV (HCV in cell culture or HCVcc), Prof Rice collaborated with Sangeeta Bhatia who was a tissue engineer in Massachusetts Institute of Technology, USA to develop a cell-based EGFP labelled reporter system (HCV-TRIPpp) that allowed for the distinction of individual HCV-infected fixed samples or live samples. This was a Fluorescence labelled image based analysis methods invented for the first time which would open a new area for HCV research. Their work was published in Nature Biotechnology, 2010 [27] (Figure 3D).

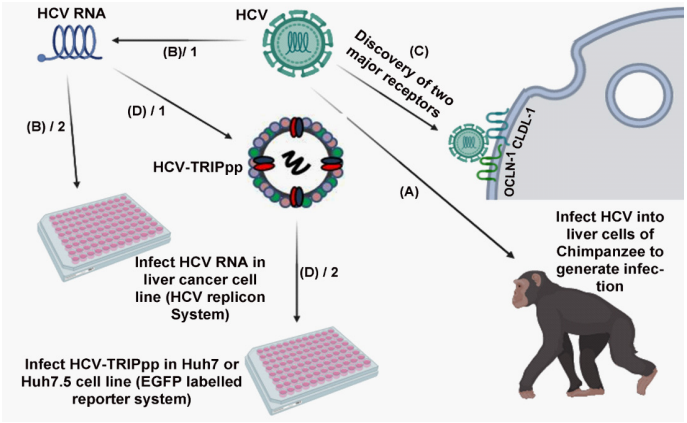

Figure 3: Schematic representation of HCV mediated infection of Chimpanzee **(A)**, generation of HCV replicon system **(B)**, discovery of two major cellular receptors for HCV entry **(C)**, generation of EGFP labelled system of HCVpp.

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