



Why No Vaccine For Hepatitis C Virus Yet?

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Introduction

In the mid 70's it was first recognized that there was a new agent responsible for post-transfusion hepatitis that was different from Hepatitis A or Hepatitis B virus. Prior to the discovery of hepatitis C virus (HCV), this viral causative agent was referred to as 'Non-A, Non-B' hepatitis or NANBH. A group of scientists at Chiron Inc had to screen countless number of clones before they were successful in finding one clone that was derived from the NANBH genome. This breakthrough occurred in the year 1989 and the newly discovered virus was termed HCV (1).

Even after two decades since its discovery, HCV still continues to be a major cause of concern and a huge burden on public health systems worldwide. An estimate by WHO suggests that a minimum of 2-3% of the world's population is chronically infected with HCV (2-4). HCV is most commonly spread by direct contact with infected blood and blood products (5). The lack of proper cleaning, disinfection of tools and equipments used in hospital and dental clinics remain a major source of virus transmission. Sharing razors or toothbrushes with an infected person, direct contact with infected blood from being pricked accidentally by a contaminated needle also spread HCV.

The amazing fact about this small RNA virus is that it can establish chronic infection in majority of the people that come in contact with it (Figure 1), despite the fact that it is detected and targeted by innate, cellular, and humoral immune mechanisms (6). Only in a very small percentage of people, HCV infection can resolve naturally (7). Chronic infection with HCV leads to development of cirrhosis (20-30 years) and eventually hepatocellular carcinoma (HCC) or liver cancer. There is no vaccine yet for hepatitis C (8). HCC is the leading cause for liver transplantation in US (9). HCV is responsible for almost 75% of all cases of HCC in Japan (10). Liver damage is not directly caused by the virus, rather is interplay between the virus and the immune system which results in the replacement of healthy liver tissue with fibrous scar tissue. Individuals with chronic hepatitis C infection frequently exhibit no symptoms. Some may report non-specific

symptoms such as fatigue, muscle aches, nausea and anorexia. Antibodies directed against several HCV proteins can be detected in chronic patients. A variety of autoimmune or immune complex-mediated diseases have also been associated with chronic HCV infection (11).

Treatment

The good news for hepatitis C is that its treatment has improved. First treatment shown to be effective against HCV involved systematic administration of interferon alpha. Through the years it has evolved to pegylated interferon, which is a stabilized version, has longer

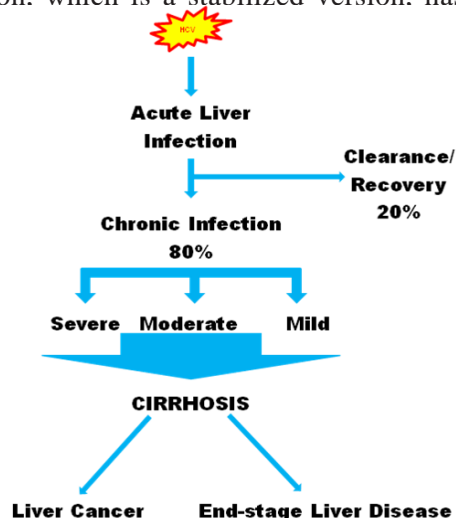


Figure 1. Acute Liver Infection is Followed by a Chronic Infection in almost 80% of the Infected Population. Chronic Infection Eventually Causes Cirrhosis Leading to Hepatocellular Carcinoma.

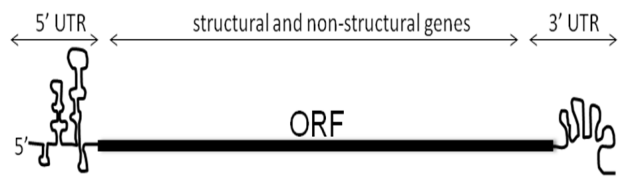


Figure 2. HCV RNA Genome: The HCV Genome Consists of a 5' Untranslated Region (UTR) Containing the Internal Ribosome Entry Site (IRES), Followed by the Genomic Coding Region and the 3' UTR. Translation of the Single Open Reading Frame (ORF) Yields a Polyprotein of ~3010 Amino Acids.

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biological half-life. Administration of pegylated interferon-alpha along with ribavirin leads to an increase in the sustained virologic response (SVR) rate. A sustained virologic response (SVR) is defined as negative HCV RNA six months after treatment. This combination therapy has indeed become the treatment of choice for hepatitis C. However, both these compounds are toxic and their administration causes adverse effects that are severe and difficult to tolerate (headache, fever, severe depression, myalgia, arthralgia & hemolytic anemia) (12).

There are wide differences in the response to treatment depending on the genotype of HCV and only 50% of the patients achieve sustained virologic responses. Currently very few treatment options are available for nonresponders. HCV genotype-1 happens to be the most resistant to interferon (IFN) therapy, whereas genotypes 2 and 3 respond much better (70-80%). The mechanism for this differential response is not known. At present, the viral genotype is the only pre-treatment predictor of treatment response. However, newer strategies are urgently needed to enhance our ability to predict treatment responsiveness. There is no doubt that further research is required to develop better antiviral with reduced toxicity.

HCV Virology

HCV is a prototype member of the Hepacivirus genus and is further classified into at least six major genotypes (1 to 6). Hepatitis C virus is a small (~55 to 65 nm), spherical, enveloped RNA virus. HCV infects the liver and replicates predominantly in hepatocytes or liver cells (10⁸ -10¹¹ copies of HCV RNA per gram of tissue). However, recent reports provide good evidence that HCV can also infect cells of extrahepatic origin, such as T and B lymphocytes, dendritic cells (myeloid and plasmacytoid lineages), gut epithelium and the central nervous system (13, 14).

The genomic organization of HCV is shown schematically in *Figure 2*. The HCV genome consists of a single-stranded, positive-sense RNA of approximately 9.6 kb. The RNA genome harbors a single ORF which is flanked by 5' and 3' nontranslated RNA segments (NTR's). Translation of the single, long ORF yields a polyprotein of ~3010 amino acids (*Figure-3*). Proteolytic processing of the polyprotein during and after translation by host and viral proteases yields at least 11 mature viral proteins (core, "F" protein, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B).

HCV Structural and Non-Structural Proteins

HCV structural proteins are located in the amino-terminal end of the polyprotein and include Core, E1, E2 and p7. An additional protein of unknown function called



Figure 3. HCV Polyprotein: The Structural Proteins (C, core; E1, envelope protein 1; E2, envelope protein 2; p7, viroporin) are Located at the Amino-Terminal end while the Nonstructural Proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are Located at the Carboxyl-Terminal End. HCV Poly-protein Undergoes Cleavages that are Directed by Host Signal Peptidases and HCV-Encoded Proteases (NS2-3, NS3-4A)

as F or ARFP or core+1 was recently identified. It results from a frameshift in the core coding region. The HCV core is a highly basic protein (15) and forms the structural component of the virus particle. The E1 and E2 are the envelope glycoproteins. The non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B are involved in viral replication. The non-structural protein 3 (NS3) is a tri-functional protein with a serine protease, an RNA helicase and NTPase activities. Its serine protease activity resides in the N-terminal (one-third) portion while the C-terminal portion possesses the NTPase and helicase activity. NS4A is a cofactor for NS3. The N-terminal portion of 4A is responsible for membrane association of the NS3-4A complex. The NS3 serine protease is responsible for cleavage at the NS3/4A, NS4A/4B, NS4B/5A and NS5A/5B junctions (16).

HCV can Antagonize Innate Immune Responses

The induction of type I IFN genes (Type I IFNs include several IFN- subtypes and a single IFN- subtype) is regulated at the step of transcription and is best understood for the IFN- promoter. Innate immune defense mechanisms activated by alpha/beta IFNs represent an essential first line of protection against viral infections. HCV has evolved strategies to antagonize innate immune responses. RIG-I (retinoic-acid-inducible protein I) is the essential PRR (pathogen recognition receptors) for HCV. RIG-I and IPS-1 (IPS-1 is also known as VISA, MAVR) signaling molecules initiate innate defenses to HCV infection. RIG-I upon binding HCV RNA undergoes changes in conformation and can then interact with IPS-1. This interaction of RIG-I with IPS-1 can signal downstream activation of IRFs (interferon regulatory factors) and NF- κ B to trigger alpha/beta IFNs. Interestingly, HCV NS3/4A protease can cleave IPS-1 (at Cys-508), resulting in the dislocation of the N-terminal fragment of IPS-1 from the mitochondria (17). By this strategy, HCV halts the expression of alpha/beta interferon thereby disrupting innate immune control mechanisms.

HCV Mutates at a Very Rapid Rate

RNA viruses replicate their genomes with the help of RNA dependent RNA polymerase (RDRP). The HCV non-structural protein 5B (NS5B) is a RNA dependent RNA polymerase (18). It initiates synthesis of complementary negative-strand RNA using the HCV



genome (positive polarity) as a template. Subsequently it generates positive-strand RNA from this negative-strand RNA template (18). NS5B lacks a "proofreading" function. Due to its high rate of error-prone replication, complex mutant swarms are generated even within a single infected individual termed as "quasispecies." ([19]). To date many NS5B polymerase inhibitors have been tested in HCV-infected chimpanzee model (20-22). However, the continuous generation and selection of resistant variants allows HCV to escape control by inhibitors/antiviral drugs.

Conclusion

For many years, the pursuit of better therapeutics was complicated by the inability to grow Hepatitis C virus in tissue culture and the absence of infectious genomes. Recent advances made in the study of HCV structure, genome and its life cycle have revealed many exciting target sites for pharmacological intervention. Rapid evolution of HCV-RNA-genomes because of error-prone replication strategy helps this virus to evade the immune response and to overcome antiviral drugs. In summary, this virus effectively evades and fools the immune system as it is always mutating at a very rapid rate. Thus, despite current advances in our understanding of HCV, many questions still remain unanswered. What is the role of HCV core, NS3, NS5A and other proteins in establishing a chronic infection? An approach where multiple, essential viral-targets are hit with an inhibitor, may yield better treatment response. A better understanding of the kinetics of the development of "quasispecies" will also help researchers to devise effective treatments for controlling virus spread. Unraveling of virus-host mechanisms should also help in moving further towards the direction of eliminating this virus. HCV establishes persistent infection in the majority of those who contract it. The ability to generate a vaccine against chronic and highly variable hepatitis C virus is a crucial and major challenge ahead.

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