Original Article

Chronic Hepatitis Virus B & Chronic Hepatitis Virus C And Clinical Cultures To Study Innate Immune Responses

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Abstract

Hepatitis virus causes inflammation of the liver, which inhibits basic liver functions. Different strains of hepatitis viruses are transmitted, infected, and treated in various methods depending on the severity and symptoms of the disease. The World Health Organization estimates around 1.5 million new Hepatitis B infections each year, with around 296 million people living with the infection as of 2019. In order to achieve optimal treatment and understanding of hepatic viruses various 2D and 3D liver cultures have been developed, in which the innate immune response of the liver can be observed through a series of scans and clinical tests. This review elucidates the advantages and disadvantages of various clinical cultures on hepatic viruses (In-Vitro & In-Vivo), and providesprevailing information on Hepatitis B and C Virusinfections in humans.

I. INTRODUCTION

The five main hepatitis viruses lead to inflammation of the liver, along with a wide range of health problems, that can potentially be fatal. The five main strains of the viral hepatitis are A, B, C, D, and E, all infect the same target organ but differ in types of symptoms, side effects, mode of entry, and treatments. The hepatitis related deaths are through liver cirrhosis, Hepatocellular Carcinoma (liver cancer), and Fibrosis, which are most commonly caused by chronic Hepatitis B and C. In the United States, over 3.5-5.3 million individuals live with the chronic strand of viral Hepatitis, in which symptoms are suppressed until severe liver damage has occurred[4]. The liver is an essential organ that is required to filter blood, detoxicate the system, and absorb vital nutrients for healthy function of the body. Hence, damage to the liver prevents quintessential functions from occurring. Hepatitis or liver damage can be caused as well by excessive alcohol intake or medication. While both chronic viruses, HCV and HBV, cause hepatitis, the manifestation, mode of transport, severity, and treatments can defer vastly.

There are several In-Vivo Model Systems to study Hepatitis. In-Vivo systems include, Primate models, Tree Shrew models, surrogate models such as Mice[6]. Essentially speaking, In-Vivo models refer to research conducted within a living organism, inthis case for human chronic hepatitis. At the same time, newly developing in-vitro models also play asignificant role in Hepatitis research. There are four quintessential in-vitro cultures that make use ofprimary human hepatocytes (PHH). PHH culturesprovide the most accurate simulation of the humanliver in an in-vitro setup when compared to that of in vivo systems. Successful in-vitro models includetwo-dimensional PHH, three-dimensional PHH, three-dimensional spheroids, and SACC PHH. Within these cultures, the hepatocyte-specific markerAlbumin is measured to investigate hepaticactivity[6]. This being said, the predominant goalbehind research in the liver infectious diseases is toidentity successful and supplementary vaccines andmedication through further understanding the diseaseas a whole, and being able to test long-term effects,side

effects, and efficiency of proposed treatments;which can potentially vary according to demography,environment, age-groups, and economical limitationswithin the research field.

II. HEPATITIS B VIRUS (HBV)

Hepatitis B is known as a "vaccine-preventable liver infection" that is caused by the Hepatitis B virus[2].This disease can manifest itself in a mild form thatlasts a couple weeks but also has a high potential tomanifest as a life-long/chronic condition; and ishighly likely to be so for unvaccinated infants lateron in their life[2]. The virus itself is spread in humansthrough bodily fluids, mainly blood contact. Such transmissions can occur through birth from aninfected mother, sexual activity with an infectedindividual, use of contaminated instruments, as wellas blood transfusions. Although not all, for those thatdo showcase symptoms, it may include fatigue,jaundice, loss of appetite, and nausea. In the cause ofchronic hepatitis viruses, this can be manifested inthe form of liver cancer or scarring of the liver tissue,which in turn disrupts proper function of the liver organ in the human body. A vaccine for this type ofHepatitis has been developed for all age groups, andit is based in the injection of HepB surface antigen, orHBsAg, which is efficiently generated artificially inthe lab; this can be given as a single stand-alonevaccine or as a combinations vaccine along withother targeted diseases[2]. The HBsAg is the surface antigen that patients are tested for to determine the presence of the HBV in the human body. The administration of the surface antigen itself creates animmune response in the human system, leading to protection against HBV through the production of neutralizing antibodies. However, it is important to note that a major limitation to the administration and development of such vaccines are the lack of economical and material resources in the field. Nevertheless, the HBsAg Vaccine has yielded as mostly successful towards preventing the infection ofthe HBV in humans[4].

Hepatitis B virus belongs to the Hepadnaviridae family, which infects and replicates in hepatocytes. Hepatocytes are the cells within the main tissue of theliver[11]. To infect, the virus binds to the Heparan Sulfate Proteoglycans and the maun receptor NTCP, which are cell surface glycoproteins. The entry of the virus occurs through endocytosis, allowing the viral DNA containing nucleocapsid to be released into the cytoplasm of the host cell. Once this occurs, the nucleocapsid disassembles and the viral genome gets into the nucleus of the cell through the nuclear pore complex. Now, with the use of the Host's RNA Polymerase II, the genome intermediate is used to generate the future Hepatitis B Virus sequences within the host's system[11].

The virion of the Hepatitis B Virus contains partially double-stranded DNA and the reverse transcriptase enzyme for the RNA intermediate to be transcribed. The icosahedral viral capsid is surrounded by a lipid envelope and contains a circular dsDNA (double-stranded DNA) genome inside[10]. The lipidenvelope includes 3 glycoproteins, S, M and L. The genome encodes for four main Open Reading Frames(ORFs). Open Reading Frames are encoding DNA sequences between the start and stop codons. Specificfor the Hepatitis B virus, the ORFs are S, C, P, and Xand encode for 7 proteins that are also essential for the life cycle of the virus. This aids towards the structure and efficient function of the virus as a whole. These code for proteins that play a vital rolein performing the essential functions within the cell and to supplement continuous infection of the virus tothe host. The quintessential proteins involved in this process are HBcAg/AbeAg, a large protein consistingof 800 amino acids, and a protein that initiates general cell functions. These are coded by ORF S, C, P, and X respectively[2]. Clinical treatments for the Hepatitis B virus include a variety of immune modulator drugs and antiviral drugs. The immune modulator drugs are given as vaccination shots that boost the immune system, potentially terminating/preventing the Hepatitis B virus, however, most are in the clinical developmental stage. On the other hand, the antiviral drugs terminateor slow down the reproduction of the viral sequence within the host system, which can reduce the inflammation of the liver. In chronic conditions, such as Hepatitis B, antiviral drugs may need to be taken for an extended duration of time, in order to reduce the inflammation of the liver to an appropriate level for the host to survive. A few main drugs include entecavir, tenofovir, and lamivudine that supports theliver and slows down the damage. Research for a complete cure is still being developed, given the limitations in clinical trials, resources available for this disease and the difficulty to target the viral cccDNA, due to its location within the nucleus of the cell . In-Vivo models have demonstrated to be very useful for Hepatitis B research,

including: Chimpanzees, the only primate model available for HBV research, vaccines for human HBV were derived through the discovery of long-term protectiveimmune responses in Chimpanzees, Tree Shrews, the only non-primate species available for HBV research,which has been found to be susceptible towards similar strands to the human HBV, and surrogate models, such as American Marmota Monax, that have led to more efficient and simplified opportunities for research in the Hepatitis B field[6]. In general, the main limitations to this type of Hepatitis, given the severity and chronic characteristics, are the necessary resources to successfully test long-term responses and appropriate treatments based on complications and population type.

III. **HEPATITIS C VIRUS** (HCV) Hepatitis C virus is also a type of virus that leads to the inflammation of the liver organ in the humansystem, caused by the Hepatitis C Virus strand.Similar to Hepatitis B, the C type can also vary fromshort term illnesses to long-term chronic diseases inhumans[3]. However, the main difference is that thereis no vaccine to prevent Hepatitis C, as there is suchfor HBV. The main method of transmissions of HCV is through contact with infected blood, throughchildbirth, use of contaminated instruments such asneedles. This being said, the main symptoms ofhepatitis C virus include, loss of appetite, fever, fatigue, abdominal pain, joint pain, and nausea[3]. The Hepatitis C virus is an enveloped, positive-strandRNA virus that consists of glycoproteins within the envelope, lipid membranes, and nucleocapsid containing the single-stranded RNA viral genome[5]. The envelope of the virus consists of twoglycoproteins, E1 and E2 glycoproteins. The E2 protein protects the E1 protein from the immunesystem, enabling successful host-receptor binding,membrane fusion, and ultimately assembly of the viral genome. Furthermore, the lipid membrane ismostly composed of cholesterol, cholesterol esters, and other lipid-like substances in the virus. It hasbeen found that the cholesterol in the lipid membraneof the virus enables the entering and assembly of the infection cell and system as a whole. Followed by the capsid or core, that plays a role in protecting the viralRNA, and are made by the Hepatitis C core protein.These proteins interact with each other in order to reach successful assembly, as the HCV RNA and envelope E1 protein receive signals to bind to the surroundings. The positive RNA single strand can be used for both translation and transcription given the presence of the RNA genome, along with an internal ribosomal entry site that regulates the entry and binding of the viral genome to the translation factors of the host. The HCV virus strand contains ten major proteins that play a role in efficient host cell infection. The first protein that comes across as essential is the Core protein, which ensures the formation of a viral capsid, including the structure to protect the viral RNA genome. The core protein is thefirst protein to get translated during the process of translation. This is followed by the E1 and E2 glycoproteins that play a role in the replication cycle along with the binding of the viral genome to the hostcell. Next in line is the P7 protein, that is also involved in the viral assembly and release from the virus to the host cell. Then, nonstructural proteins 2, 3, 4 and 5 participate in the host-cell interaction, enzyme activity, cofactors, and in general, catalysis of essential processes and requirements for the life cycle. There are nine brief steps involved in the life cycle of the Hepatitis virus. Starting with the binding of the virus to the surface of the host cell through the Low Density Lipoprotein Receptor (LDLr) andHeparan Sulfate Proteoglycans (HSPGs). A few notable receptors that intervene with the initial steps of HCV infection include Tetraspanin CD81, tight-junction protein claudin-1, and occludin[3]. Then, this initiates the entry of the viral particle into the hepatocyte, commonly known as endocytosis. Once this occurs, the virus particle uncoats the outer layer, and is free to move within the cytosol, a liquid found inside the cell, within the endosomal vesicle. This is when the process of translation and replicationcan take place. The translation process takes place along with the ribosomal subunits, in which a single polyprotein is created. Then, after the proteins are processed in the endoplasmic reticulum of the cell, RNA replication takes place. The RNA replication process is aided by the HCV nonstructural proteins. This is followed by the process of assembly, in whichthe HCV particles assemble at a specific point and are filtered based on maturation in order to be released within the cell. In the Golgi, maturation of the particles takes place, preparing its release. Finally,the multivesicular HCV viral particles approach the cell surface, in which they fuse to the cell membrane. These particles are then released into

the rest of the cell, through which the infection spreads[2].

While symptoms for Hepatitis C virus may remain suppressed or may manifest itself in diseases related to the liver, there are also extrahepatic manifestationsof the virus that could lead to other complications. Diabetes mellitus1, Glomerulonephritis2, Essential mixed cryoglobulinemia3, Porphyria cutanea tarda4, and Non-Hodgkin's lymphoma5 are all medical conditions that could be developed due to a HCV infection[3]. Hepatitis C can be managed using direct-acting antiviral (DAA) tablets, reducing the damage imposed on the liver in the human system. It has also been advised that patients at risk of chronic Hepatitis C Virus should be vaccinated with HepatitisA and B vaccines, to prevent development of further medical conditions[5].

IV. INNATE IMMUNE RESPONSES

Ideally, when such viruses attack the system, thisshould trigger the activation of the body's innateimmune response system. Which is a nonspecificdefense system that works as the first line of defenseduring any viral attack or infection. There are four types of defensive barriers that can initiate a responsein the body as a part of the innate immune system based on the types of signals received: anatomical, physiologic, endocytic/phagocytic, and inflammatorysystems.

Anatomical includes the formation of skin and mucous membranes, physiological includes changes in body temperature and pH, endocytic and phagocytic involve the proteins within the body, and inflammatory involves the body's cells to heal a wound. In the case of Hepatic viruses, a specific pathogen recognition receptor (PRRs) recognizes the strain of the hepatitis virus and triggers the innate immune response system. The innate immune response system consists of the production of interferons (IFNs) and TLR proteins that are within the JAK-STAT pathway of the immune system. A common signaling pathway for these types of pathogens begin with the TLR proteins that are located at the surface of the cell, which gets activatedin the presence of various parts of the pathogen (such as DNA, RNA, strand type, and proteins). These TLRproteins are present both in the surface and in the endosome of the cell, so that detection of the virus can be made at the initial stage of infection or during endocytosis. Subsequently, interferons are an antiviral factor that can be synthesized by any cell type and essentially interrupts the replication process of the virus. In the liver, the hepatocytes are in chargeof synthesizing the innate immune proteins for defense. With this in mind, the second signaling pathway includes the detection of double-stranded HBV particles in the cytosol of the cell. This is done using the Retinoic Acid Inducible gene I (RIG-I), which binds to the HBV dsRNA particles, essentially inhibiting/disturbing the production of essential proteins and processes. Essentially, the interferons binding to a receptor on the surface of the cell that activates the JAK-STAT pathway of the immune system, account for antiviral efficacy within the system[12].

V. IN-VITRO CULTURES: PRIMARYHUMAN HEPATOCYTES

In order to effectively simulate hepatitis infected human hepatocytes in the liver, there are four successful microfluidic liver cultures that have been adapted for research. 2D PHH cultures, 3D PHH cultures, 3D Spheroids, and Self-Assembling Co-Cultures (SACCS). The presence of active hepatic cells are determined by Immunofluorescence microscopy of Albumin production visible through the micrographs, as Albumin is a protein predominantly made by the liver. Overlaying the 2D primary hepatocytes on an extracellular matrix,enabling interactions with other proteins, simulates the physiological environment of the human liver. Anadvantage to this model is that materials and setups are commercially available for labs, and aren't time consuming to assemble and monitor changes. However, a disadvantage to the 2D cultures is the number of days the culture can be monitored without physiological changes to the cells, resulting in inaccurate data collection. Subsequently, the 3D primary hepatocytes culture provides an accurate phenotype of the liver and hepatocytes, and sustains the PHH for a longer duration for testing. Additionally, 3D Spheroids are three-dimensional cellstructures that mimic various tissues and tumors, and have been developed to investigate PHH as well[8]. Finally, Self-Assembling Co-Cultures (SACCS) are a3D microfluidic liver culture that provides the most efficient liver culture, in which hepatocytes have the ability to retain their structure and sustain the infection for up to 40 days[7].

VI. CONCLUSION

All in all, the chronic hepatitis virus B and C are diseases that are still being researched about, and have treatments that are being improved and developed to better suit various populations. Along with political and economical limitations, ethical and moral values also need to be considered when carrying out clinical trials for such viruses.

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AUTHOR CONTRIBUTION STATEMENT

Katherine Emi wrote the main manuscript text, including the abstract and formatting. Dr. Ortega edited and mentored throughout the manuscript. Most of the research was done from publications of Dr. Ortega and her team's achievements on immunology and virology research. All authors reviewed the manuscript.

ADDITIONAL INFORMATION

The authors declare no competing interests.