Characterization of Hepatitis C Virus (HCV) and HCV E 2 Interactions with the Low- Density Lipoprotein Receptor (LDL r) and CD 81

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LIST OF ABBREVIATION

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Au	Australia antigen
BCP	Basic core promoter
bDNA	Branched deoxyribonucleic acid
cDNA	Complementary deoxyribonucleic acid
CTLs	Cytotoxic T lymphocytes
DEMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
EIA	Enzyme immunosorbient assay
ELISA	Enzyme linked immunosorbent assay
FCS	Fetal calf serum
Fig	Figure
HAA	Hepatitis associated antigen
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma"
HCV	Hepatitis C virus
HCVAb	Antibody for hepatitis C virus
HDV	Hepatitis delta virus
HEV	Hepatitis E virus
HIV	Human immunodeflcience virus
IgG	Irnmunoglobulin Gamma
IgM	Immunoglobulin M
LDL	Low density lipoprotein
LHBs	Large hepatitis B surface antigen
MHC	Major HistocompatibiUty complex
NA,NB	NonH-A, non-B hepatitis
NK	Natural killer cell
ORF	Open reading frame
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol

Pos	Positive
PreC	Precore
RIBA	Recombinant immuno blot assay
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
RTD-PCR	Real-time detection polymerase chain reaction
Tc	T cytotoxic cell
Th	T helper cell
Ts	T suppressor cell
TTV	Transfusion transmitted virus
UTR	Untranslated region
VSV	Vesicular stomatitis virus

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INTRODUCTION

Hepatitis as an entity has been recognized for thousands of years. The description of jaundice, i.e., hepatitis, dates to ancient Chinese writings, and the first European reference dates to the third century A.D. Recognition that hepatitis has an infectious etiology has been in the modern literature since early in this century ***(Cockayne, 1912)***, and the terms hepatitis A and B were first used in 1947 ***(Havens, 1947)***. However, proof that viruses are responsible for this disease was first published in 1968 with the description of hepatitis B virus (HBV) particles in nature ***(Bayer et al, 1968).***

Hepatitis is an inflammation of the liver. Clinically it is characterized by jaundice, abdominal pain, liver enlargement and sometimes fever. It is usually caused by viral or non viral agents but also may be a result- of alcoholics. Viral hepatitis is a systemic infection affecting the liver ***(Feinstone et al, 1973)***. Five different human hepatitis viruses have been recognized and characterized in detail. The five established agents are hepatitis A virus (HAV) (formerly called infectious hepatitis) ***(Feinstone et al, 1973),*** hepatitis B virus (HBV) ***(Dane et al, 1970),*** hepatitis C virus (HCV) (formerly called parenterally transmitted non-A, non-B hepatitis) ***(Choo et al, 1989),*** hepatitis D virus (HDV) (delta hepatitis) ***(Wang et al., 1994)***, and hepatitis E virus (HEV) (enterically transmitted non-A non-B) ***(McCaustland et al, 1991).*** Two additional viruses designated GB virus-C/ hepatitis G virus ***(Deinhardt et al, 1967)***, and transfusion transmitted virus (TTV) ***(Nishizawa et al, 1997)***; however, these blood-borne agents have not been established as human hepatitis pathogens. All human hepatitis viruses are RNA viruses except for hepatitis B, which is a DNA virus; despite differences in their genomes, molecular structure, and virus classification, they all target the liver primarily and cause a characteristic necro inflammatory process, hepatitis. While all of them can cause acute hepatitis, only HBV, HDV, and HCV cause chronic hepatitis.

The hepatitis viruses differ widely not only in their respective molecular structures but also in their modes of transmission and clinical features; however, common themes exist among all hepatitis viruses ***(Kevin and Jules, 2002)***.

Non- hepatotropic agents, such as yellow fever, Epstein Barr (EBV) and cytomegalovirus (CMV), may cause viral hepatitis. Hepatitis A and E viruses are enterically transmitted (via oral ingestion of fecal material from infected patients), whereas hepatitis B, C, and D viruses are transmitted by parenteral (via exposure to infected body fluids) exposure. The enteric ally transmitted hepatitis viruses generally produce a self-limiting hepatitis followed by complete recovery. The parent rally transmitted hepatitis viruses can persist as chronic infection in the form of chronic hepatitis and' eventual development of cirrhosis and hepatocellular carcinoma ***(Feinstone et al, 1973a; Morgan and Smallwood, 1996)***.

Hepatitis C virus (HCV), a member of the Flavivridae family of viruses, is a major cause of chronic hepatitis and hepatocellular carcinoma. The HCV genome is a positive- strand9.6-kb RNA molecule consisting of a single open reading frame, which is flanked by 5 and 3 untranslated region. The HCV 5 – untranslated region contains a highly structured internal ribosome entry site. The HCV open reading frame encodes a single polyprotein that is 3.008-3.037aa in length and is post-translationally modified to produce at least ten different proteins: core, envelope proteins (E1 and E2), P7, and non structural proteins (NS2, NS3, NS4a, NS4B, NS5A, and NS5B).Despite considerable advances in understanding the function of these proteins, the basic mechanism(s) of HCV replication remains unclear. The recent development of HCV culture and expression of HCV proteins in stably transfected human cells has facilitated the analysis of the role of cellular pathways required for HCV replication (***Virology Journal 2009, 6:13***).

TISSUE CULTURE

There are four basic requirements for successful cell culture. Each of these will be briefly reviewed in this introduction. The first necessity is a well-established and properly equipped cell culture facility. The level of biocontainment required is dependent on the type of cells cultured and the risk that these cells might contain, and transmit, infectious agents. For example, culture of primate cells, transformed human cell lines, mycoplasma-contaminated cell lines, and non tested human cells require a minimum of containment facility. All facilities should be equipped with the following: a certified biological safety cabinet that protects both the cells in culture and the

worker from biological contaminants; a centrifuge, preferably capable of refrigeration and equipped with appropriate containment holders that is dedicated for cell culture use; a microscope for examination of ceil cultures and for counting cells; and a humidified incubator set at 37°C with 5% CO2 in air. A 37°C water bath filled with water containing inhibitors of bacterial and fungal growth can also be useful if warming of media prior to use is desired. Although these are the basic requirements, there are numerous considerations regarding location of the facility, airflow, and other design features that will facilitate contamination-free culture. If a new cell culture facility is being established, the reader should consult facility requirements and laboratory safety guidelines that are available from your institution's biosafety department or the appropriate government agencies.

The second requirement for successful cell culture is the practice of sterile technique. Prior to beginning any work, the biological safety cabinet should be turned on and allowed to run for at least 15 min to purge the contaminated air. All work surfaces within the cabinet should be decontaminated with an appropriate solution; 70% ethanol or isopropanol are routinely used for this purpose. Any materials required for the procedure should be similarly decontaminated and placed in or near the cabinet. This is especially important if solutions have been warmed in a water bath prior to use. The worker should don appropriate personnel protective equipment for the cell type in question. Typically, this consists of a lab coat with the cuffs of the sleeves secured with masking tape to prevent the travel of biological contaminants and Latex or vinyl gloves that cover all exposed skin that enters the biosafety cabinet. Gloved hands should be sprayed with decontaminant prior to putting them into the cabinet and gloves should be changed regularly if something outside the cabinet is touched. Care should be taken to ensure that anything coming in contact with the cells of interest, or the reagents needed to culture and passage them, is sterile (either autoclaved or filter-sterilized).

A third necessity for successful cell culture is appropriate, quality controlled reagents and supplies. Unless otherwise specified in the protocols accompanying your cells of interest, any source of tissue-culture-grade reagents should be acceptable for most cell culture purposes. Similarly, there are numerous suppliers of the plastic ware needed for most cell culture applications (i.e., culture dishes and/or flasks, tubes, disposable pipettes). Two cautionary notes are essential. First, sterile culture dishes can be purchased/as either tissue culture