cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Engineered Livers for Infectious Diseases

Nil Gural,[1,2] Liliana Mancio-Silva,[2,3] Jiang He,[2,3] and Sangeeta N. Bhatia[2,3,4,5,6,7]

[1]Harvard-MIT Department of Health Sciences and Technology, Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Boston, Massachusetts; [2]Koch Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, Massachusetts; [4]Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts; [5]Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts; [6]Broad Institute, Cambridge, Massachusetts; and [7]Howard Hughes Medical Institute, Chevy Chase, Maryland

SUMMARY

This article discusses existing 2-dimensional and 3-dimensional liver models and their applications toward studying hepatotropic pathogens. The importance of selecting the most appropriate models and readout modalities to answer specific scientific questions is emphasized.

Engineered liver systems come in a variety of platform models, from 2-dimensional cocultures of primary human hepatocytes and stem cell–derived progeny, to 3-dimensional organoids and humanized mice. Because of the species-specificity of many human hepatropic pathogens, these engineered systems have been essential tools for biologic discovery and therapeutic agent development in the context of liver-dependent infectious diseases. Although improvement of existing models is always beneficial, and the addition of a robust immune component is a particular need, at present, considerable progress has been made using this combination of research platforms. We highlight advances in the study of hepatitis B and C viruses and malaria-causing *Plasmodium falciparum* and *Plasmodium vivax* parasites, and underscore the importance of pairing the most appropriate model system and readout modality with the particular experimental question at hand, without always requiring a platform that recapitulates human physiology in its entirety. (*Cell Mol Gastroenterol Hepatol 2018;5:131–144; https://doi.org/10.1016/j.jcmgh.2017.11.005*)

Keywords: Liver; Liver Models; 3D; in vitro; in vivo; Hepatotropic; Pathogen; HBV; HCV; Malaria; Falciparum; Vivax.

The liver is the largest internal organ in the body, and performs vital and diverse functions in metabolism of carbohydrates, proteins, and lipids; bioproduct synthesis; immunologic processes; and detoxification. Although many viruses, parasites, and bacteria specifically target the cells of the liver, the liver is also exposed to blood-borne pathogens that circulate systemically, or that are derived from the gut,[1] because of its location at the convergence of the hepatic artery and portal vein.

Most liver pathogens specifically target the most abundant cell type in the liver, the hepatocyte, for completion of their life cycles or developmental stages. These include hepatitis viruses and *Plasmodium* protozoan parasites (Figure 1), which together account for an enormous burden on human health. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infect the livers of more than 350 million people worldwide, and are the main causes for chronic liver diseases, such as liver cirrhosis and hepatocellular carcinoma.[2] *Plasmodium* parasites, which cause malaria, result in more than 200 million infections annually[3] and require asymptomatic development in the liver before initiating fevers associated with blood stage infection. Other hepatropic pathogens, including several viruses and bacteria that cause systemic infection, can also target the liver and cause severe liver damage (Table 1).

In addition to hepatocytes, the liver is also populated by other cell types, such as Kupffer cells, liver sinusoidal endothelial cells, cholangiocytes, and stellate cells, some of which can be targeted by pathogens. For example, human cytomegalovirus can infect bile duct epithelia cells and stromal cells,[4] whereas dengue virus can replicate in Kupffer cells[5] and hepatocytes.[6] Aside from being a site for massive pathogen amplification, the liver also hosts such pathogens as *Entamoeba hystolytica*, a protozoan parasite that travels from the gut via portal vein, invades the liver parenchyma, and remains extracellularly, forming amoebic liver abscesses.

To study the mechanisms of pathogen-host cell interactions and to develop novel therapeutics against liver pathogens, robust model systems that can faithfully replicate human hepatotropic infections are needed. Human hepatoma-derived cell lines have been widely used to study the biology of hepatotropic pathogens and to test candidate drugs and vaccines. However, because of their uncontrolled proliferation, abnormal liver-specific functions,[7,8] or the stringent host dependence of some human

Abbreviations used in this paper: 2D, 2-dimensional; 3D, 3-dimensional; EBOV, Ebola virus; HBV, hepatitis B virus; HBC, hepatitis C virus; HLC, hepatocyte-like cells; iHLC, induced pluripotent stem cell–derived hepatocyte-like cells; LASV, Lassa virus; MPCC, micropatterned coculture system; PCR, polymerase chain reaction; SACC, self-assembling coculture.

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2017.11.005

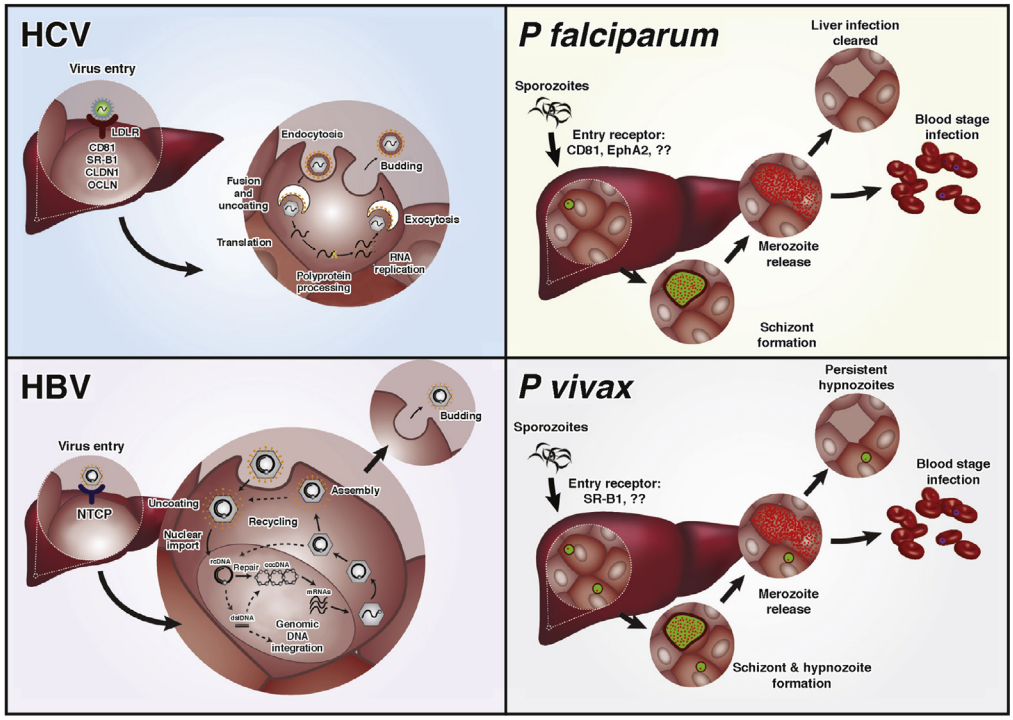

Figure 1. Life cycles of 4 major human hepatotropic pathogens. HCV is a single-stranded, positive-sense RNA virus that belongs to the Flaviviridae family. Initial viral attachment to the hepatocyte membrane is mediated through glycosaminoglycans and the LDL receptor. Interactions with other host factors CD81, scavenger receptor class B member 1 (SRB1), claudin 1 (CLDN1), occludin (OCLDN), and possibly other molecules, such as CLDN9, CLDN6, EphA2, and epidermal growth factor receptor, are required for cell entry. Clathrin-mediated endocytosis of the virus is followed by fusion of the viral and endosomal membranes, resulting in the release of nucleocapsid into the cytoplasm. Positive-strand genomic RNA is released into the cytosol on uncoating of the viral nucleocapsid, which initiates synthesis of the HCV polyprotein. Host cell lipid synthesis pathways are tightly linked to the later stages of assembly and virus release. HBV is a DNA virus that belongs to the family Hepadnaviridae. HBV enters the hepatocyte via the sodium/bile acid cotransporter NTCP.[23] After uncoating, the partially relaxed double-stranded circular viral DNA (rcDNA) is directed to the nucleus where viral DNA lesions are repaired by the host machinery, converting into covalently closed circular DNA (cccDNA), which serves as a template for viral RNA production. Five transcripts are made that encode envelope, core and X antigens, viral polymerase, and pregenomic RNA (pgRNA). pgRNA can be reverse transcribed into rcDNA, which is assembled with the viral capsids and released from the host cell. During reverse transcription of pgRNA double-stranded linear (dsl) DNA can be formed and are capable of integration into human chromosomes. *Plasmodium falciparum* and *Plasmodium vivax* are apicomplexan parasites. *Plasmodium* sporozoites are deposited into the human skin via bite of an infected Anopheles mosquito and travel to the liver where they invade hepatocytes. CD81[38,148] and EphA2[106] for *P falciparum*, and more recently SR-B1[134] for *P vivax*, have been implicated as required entry factors. On invasion of hepatocytes, parasites differentiate and divide by schizogony to form thousands of progeny, merozoites, which are released into the bloodstream where they can cyclically invade red blood cells, initiating the blood stage of the disease. *P vivax* has an additional, unique aspect of its liver development where a subset of the parasites, called hypnozoites, remain dormant and can reactivate weeks to years after the initial infection to reinitiate disease.

hepatotropic pathogens, immortalized cell lines do not always fully recapitulate the entire pathogen life cycle. Animal models are also not alternatives because many human-tropic species can only infect hepatocytes of human origin. Human hepatocytes are thus considered the gold standard cell type to study the biology of human hepatotropic pathogens and pathogen-host interactions, yet conservation of the polarized morphology and functions of hepatocytes *ex vivo* is challenging.

To develop systems that closely recapitulate human liver biology and support hepatotropic infections, tissue engineering tools have been applied to create 2-dimensional (2D), 3-dimensional (3D), and humanized mouse systems by using a combination of cell lines, primary human hepatocytes, or stem cell–derived cells with various extracellular matrix manipulations (Table 1). The available systems are capable of modeling some, but not all, aspects of the shared pathogen-host interaction, thus researchers should carefully select a model that is best suited to the specific question being investigated. In this review, we summarize key aspects of each platform, their advantages and disadvantages, and discuss biologic insights gained using models of liver infections, with a focus on HBV and HCV viruses and the major species of human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax* (Figure 1). For more technical details on the assembly of various engineered liver model systems we recommend a collection of recent review articles.[9–12]