Doi: 10.4274/vhd.44366
Viral Hepatitis Journal 2015; 21(3): 65-71

Animal Models for Hepatitis C Infection Studies

Hepatit C Enfeksiyonunda Hayvan Modeli Çalışmaları

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ABSTRACT

Hepatitis C virus (HCV) infection is among the main causes of cirrhosis and hepatocellular carcinoma all over the world. Despite common use of direct acting antiviral agents, treatment in chronic HCV hepatitis remains inadequate; and HCV is responsible for more than 300,000 deaths annually due to hepatic reasons. Currently, there is not a vaccine available to prevent HCV infection. Since its discovery, our knowledge about HCV has grown substantially with the help of animal studies. Development of appropriate animal models has great importance for research on HCV and for development of vaccine and medications. Limitations in studies on HCV are mostly because appropriate animal models cannot be found due to restraints arising from selective sensitivity of HCV between humans and primates. Chimpanzees are the best models for studies on innate or acquired host immune responses and HCV infection. The chimpanzee model has played an important role both prior to and after the discovery of HCV, as the etiological agent of non-A, non-B hepatitis (NANBH). Chimpanzees have been irreplaceable for proving NANBH is an infectious disease caused by a separate agent; furthermore they provided great benefits as an *in vivo* system for identification trials and for obtainment of sufficient material to be used in biological amplification of the virus. Large amounts of plasma have been obtained from chimpanzees for molecular cloning of HCV genome. Recently, the focal point of the studies have been the animal modification for the purpose of letting the HCV entry, replication and infectivity. In this study, we have discussed the proper animal models, in which there are chimpanzees, tupaia, mouse and rats for HCV studies. In addition to this, we have also aimed to investigate the advantages and disadvantages of each model in the model design process.

Studies on animal models have critical significance in that they cast light on the full comprehension of the HCV infection, and form the foundations of future treatments and possible prevention measures.

Keywords: Animal models, hepatitis C, mouse, rat, tupaia

ÖZ

Hepatit C virüs (HCV) enfeksiyonu tüm dünyada, siroz ve hepatosellüler karsinomanın başlıca nedenlerinden biridir. Direkt etkili antiviral ajanların yaygın kullanımına rağmen kronik HCV hepatiti tedavisi yetersiz kalmakta ve HCV her yıl 300,000'den fazla kişide hepatik nedenlerle ilişkili ölümlerden sorumlu olmaktadır. Günümüzde HCV enfeksiyonundan korunmada, mevcut bir aşı bulunmamaktadır. İlk keşfinden bu yana, HCV hakkındaki bilgilerimiz, hayvan çalışmalarının yardımı ile, ciddi şekilde artmıştır. Uygun hayvan modellerinin geliştirilmesi; HCV araştırmasında, aşı ve ilaç çalışmalarında büyük öneme sahiptir. HCV çalışmalarındaki kısıtlılık çoğunlukla, insan ve primatlardaki seçici duyarlılık sınırlamasına bağlı olarak, uygun hayvan modellerinin bulunamamasından kaynaklanmaktadır. Şempanzeler, doğuştan var olan ya da sonradan kazanılan konak immün cevapları ve HCV enfeksiyonu ile ilgili çalışmalar için en iyi modeldir. Şempanze modeli, non-A, non-B hepatitlerin (NANBH) etyolojik ajanı olan HCV'nin keşfi öncesinde ve sonrasında önemli rol oynamıştır. NANBH'nin aslında, bulaşıcı bir ajana bağlı gelişen bir enfeksiyon hastalığı olduğunun kanıtlanmasında vazgeçilmez olan şempanzeler aynı zamanda; identifikasyon denemelerinde ve virüsün biyolojik amplifikasyonunda kullanılmak üzere yeterli materyalin elde edilmesinde *in vivo* sistem olarak çok yararlı olmuşlardır. HCV genomunun moleküler klonlaması için kronik enfekte şempanzelerden çok miktarda plazma elde edilmiştir.

Son yıllardaki araştırmalar HCV girişi, replikasyonu ve bulaşına izin vermek için hayvanların modifikasyonuna yoğunlaşmıştır. Biz bu yazıda, HCV çalışması için, şempanze, tupaia, fare ve ratların olduğu uygun hayvan modellerini ve model dizaynında her bir modelin avantaj ve dezavantajlarını tartışmaya çalıştık. Hayvan modellerindeki araştırma, gelecekteki tedaviler ve hastalığın önlenmesine temel oluşturması için HCV enfeksiyonunun tam olarak anlaşılmasında, kritik öneme sahiptir.

Anahtar Kelimeler: Hayvan modelleri, hepatit C, fare, rat, tupaia

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Viral Hepatitis Journal, published by Galenos Publishing.

Introduction

Hepatitis C virus (HCV) is a single-stranded ribonucleic acid (RNA) virus with positive polarity, belonging to the family Flaviviridae. According to the estimates from the World Health Organization, the virus affects nearly 3% of the population of the world. Also, there are over 170 million humans world wide who are chronic carriers of HCV, and 3-4 million people get infected with HCV each year (1,2,3). HCV primarily infects hepatocytes, causing acute hepatitis. Presumably in 15% of these acute infections, the virus is cleared without any medical intervention, while chronic hepatitis C develops in the rest. Nearly 30% of the chronic hepatitis C cases develop to cirrhosis, and 20% of them progress to hepatocellular carcinoma. Therefore, the infection of HCV is a global health problem that requires constant research in order to gain an insight on the viral infection and to develop strategies for medication (4).

Attempts to understand the complexities of HCV regarding entry to the cell, life-cycle and replication have been restrained due to lack of proper live models to be used as experimental hosts for the virus (2). Advances in basic and clinical studies on HCV infection and its association with acute and chronic liver disease demand examples of *in vitro* and *in vivo* models (5).

Development of animal model systems has provided an invaluable source for research on human viral hepatitis. Most of our knowledge, including the discovery that HCV was the most important agent in non-A, non-B hepatitis (NANBH), comes from the studies conducted on chimpanzees. The first evidence that NANBH is an infectious disease occurring associated with an infectious agent, has been the successful demonstration of the infection in chimpanzees with human NANBH. Furthermore, chimpanzees have provided an *in vivo* system in obtaining sufficient material for identification trials of the suspected virus (6).

Human beings and other primates are the sole organisms that are known to naturally host the infection of HCV. Disease research using chimpanzees is limited due to cost and ethical concerns (2). Within the last decade, only a few small animal models have been developed as an alternative to chimpanzees; other primates have not been found susceptible to the infection (5). A general overview of HCV animal models and procedures in HCV studies, and development of drug and vaccines are presented in Table 1 (5,6).

In this study, the present situation of the HCV animal models is presented in brief. Benefits and shortcomings of animal models, and their future role in HCV studies are discussed.

Animal Models

Chimpanzees for Animal Model of Hepatitis C Virus

Chimpanzees are closely connected with the history of HCV discovery (7). They are innately susceptible for HCV infection, thus, their usage for HCV researches has contributed to our knowledge of this virus (8). These animals can be chronically infected by viruses by using several sources like inoculum (7). Except for research of natural infections that occur in humans, experimental infection of chimpanzees has played a very large role in discovering HCV as well as being very valuable in understanding host-virus interactions and preclinical analysis of antiviral strategies (9). Although clinical courses of HCV infections in chimpanzees and humans are not similar, HCV researches on chimpanzees have provided important information about both innate and humoral immune responses on the mechanism of the infection, replication and the course of the infection (2,7). Aminotransferase levels in chimpanzees infected with HCV are seen to be elevated and in liver biopsies, necroinflammatory changes are observed after an acute infection. However, the genomes that have cell culture adaptive mutations in chimpanzees were found to be highly attenuated which emphasizes the inconsistencies and limitations for the biological importance of the *in vitro* systems (9).

When more than 98% genetic similarity between chimpanzees and humans was considered; chimpanzees demonstrate differences from humans because of the mild clinical course of infection although HCV can use host factors in chimpanzees as effectively as in humans (8,9). It has been reported that cirrhosis or fibrosis did not develop in chronically infected chimpanzees, and they had more moderate symptoms. However, it has been reported that hepatocellular carcinoma due to HCV had developed in only one chimpanzee. Another difference is the interferon (IFN) treatment being ineffective, which is shown with the continuance of viral load despite the use of this agent (2,7).

Chimpanzees are substantially useful in testing various antiviral drugs (7). Due to the up-to-date clinical studies and the use of new HCV protease inhibitors, the gains in the treatment of HCV-infected chimpanzees could lead to the encouragement of clinical studies performed on humans in regards to antiviral agents where pegylated IFN and ribavirin are used together (2). However, it could not be possible to determine a significantly safe and effective drug for humans until today (7).

There are numerous evidences indicating the great role of T cell responses in preventing HCV infection and in viral clearance. It has been demonstrated that memory T cells played a role in activation of intrahepatic natural killer (NK) cells and type 1/2 IFN production and, additionally, they inhibited recurrent infection of HCV (10).

Chimpanzees form a valuable animal model for studies of active immunity and research on congenital and cell-mediated antiviral activity mechanisms. It is the only animal model that allows widely evaluating the efficacy of potential vaccines against HCV (2,7). There were difficulties in the evaluation of the results of vaccination studies in chimpanzees infected with HCV, in some of them the genotypes were heterogeneous, NK and T-cell responses reduced after the error-prone RNA polymerase, which provides development of mutations that are resistant to neutralizing antibodies, and gpE2 and CD81 interaction were among these difficulties. With all of these difficulties, some important information can still be obtained from the therapeutic and prophylactic vaccination studies (2). It has been demonstrated in a meta-analysis of HCV vaccine efficacy in chimpanzees that therapeutic vaccines with structural proteins provided better T cell stimulations than vaccines with only non-structural proteins and they were more effective (11). However, successful data must be carefully interpreted in vaccinations, since in most studies, reduction of the clinical disease is taken as a basis instead of the virologic response continuing as an outcome (2).

The mechanism of preventive vaccination generally involves the formation of neutralizing antibodies. These neutralizing antibodies are present along with high HCV titers in HCV suggesting that the presence of these antibodies does not prevent *in vivo* entry of HCV into the cells. After the infection is cured, the neutralizing antibody

levels also tend to decrease; this indicates the absence of memory response or the capacity for preventing a recurrent infection. The focus of more recent studies has been on forming reliable T-cell (CD4+ and CD8+) response for the purpose of preventing the infection with being exposed to the virus (2). A vaccination strategy that is prophylactic in chimpanzees using adenoviral vectors and plasmid DNA applied electroporation coding the nonstructural region of HCV was developed by Folgori et al. (12). By stimulating T-cell response giving cross-reactivity, the chimpanzees overcame the infection upon encountering the virus which is different from the amino acid level below about 13%. Studies regarding vaccinations in chimpanzees are ongoing (2).

Starting from the first studies as the means of determining the cause of HCV, as NANBH, until 2013, chimpanzees formed the most important *in vivo* model for studies related to immune response in the creation of infectious cDNA clone of the virus (8). HCV does not seem to be capable of continuously causing infections in nonhuman primates except for chimpanzees, even though the HCV life cycle is repeated in hepatocytes derived from induced pluripotent stem cells (iPSC) obtained from pig-tailed macaques (2). Studies of HCV infections on chimpanzees have improved our knowledge of essential immune responses for effective clearance of viral infection, however, this model has some limitations (Table 1). In fact, due to the biological variability between individuals and group animals, data from studies of chimpanzees are quite variable and explicating these data is mostly difficult. Additionally, while chronic infections occur only in 30-40% of infected animals; in humans, this rate reaches up to 85% (7).

In conclusion, limited procurement, cost of acquisition and care of experimental animals as well as ethical concerns are the basic handicaps for the use of these animal models. Recently, the use of chimpanzees for behavioral and biomedical researches is prohibited by the National Institutes of Health (NIH) following the recommendations of the institute of medicine and it is also prohibited in Europe after new EU Directive (2010/63) (7,8,13).

Tupaia

Tupaia belangeri indigenous to Southeast Asia are squirrel-like non-rodent mammals. It has been suggested that tupaias were susceptible to various human viruses, such as herpes simplex, rotavirus and HBV and they could be infected with serum-derived HCV (8,9). Replication of active hepatitis C and synthesis of virion in primary tupaia hepatocytes have been demonstrated by Zhao et al. (14) in 2002. In this study, they implanted primary tupaia hepatocytes in the medium and infected them with serum or

Tablo 1. Animal models for hepatitis C virus studies (6,7,9)

	Human	Chimpanzee	Tupaia	uPA-SCID	FRG	AFC8-huHSC/Hep	Rosa26-Fluc mice	Rat-immunotolerized
Genetic manipulation	No	No	No	Yes	Yes	Yes	Yes	Limited
Entry/infection	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Viral production	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Viremia levels	High	High (low than Human)	Low	High	High	No	No	Low
Liver disease	Hepatitis, cirrhosis, fibrosis, HCC	Acut hepatitis, Milder than humans	Hepatitis, cirrhosis, fibrosis	No	No	Hepatitis, fibrosis	No	No
Effective IFN treatment	Yes	No	Unknown	Yes	Yes	No	No	Unknown
Direct acting antiviral agents	Yes	Yes	Unknown	Yes	Unknown	No	No	Unknown
Vaccine development	Yes	Yes	Unknown	No	No	No	No	No
Cost	High	High	Medium	Low	Low	Low	Low	Medium

UPA-SCID: Urokinase-type plasminogen activator-Severe chimeric combined immunodeficient, IFN: Interferon

plasma taken from humans infected with HCV. It was confirmed by the detection of a negative RNA strand with infection and active replication reverse transcription polymerase chain reaction, and the secretion of viral particles in the medium. During 14 days following implantation, HCV RNA could be detected. It was seen that the produced enveloped virions had resistance to destruction by ribonucleases and could infect the tupaia hepatocytes which were not infected previously (14).

In a study performed in 2010, HCV-infected tupaias were followed up for three years after inoculation with hepatitis C. During an acute infection, mild inflammation and viremia and, then, steatosis, cirrhotic nodules and tumorigenesis in the liver were observed in the animals. Moreover, when serum was collected from the infected tupaias and inoculated to healthy tupaias, an acute infection developed, which in turn showed the active replication and transmission potential of HCV (15).

HCV can use tupaia orthologs of CD81, scavenger receptor class B type I (SR-BI), claudin (CLDN) and occludin (OCLN) for entering to tupaia hepatocytes (8). It has been demonstrated that HCV entry factors of tupaia like CD81, SR-BI, claudin-1 (CLDN-1) and OCLN enabled HCV pseudoparticles or HCV obtained from cell culture (HCVcc) to enter primer tupaia hepatocytes or human or murine cells that were laid out to express these host factors (16,17). Blocking HCV entry by CD81 or SR-BI inhibition may be useful for determining the potential goals of drugs that will be developed to prevent virus entry in humans. However, the rate of infection is low in tupaias and HCV viremia is transient and moderate. This leads to mild hepatitis in acute phase of the infection; the compatibility of host environment with HCV replication is low in tupaias (8,9).

Although they are promising, their limited availability, maintenance costs, inability to generate chronic phase of HCV, absence of markers specific to tupaia to evaluate HCV-host interaction, and difficulties in mating these tupaias are still going on to limit their usage in studies of HCV pathogenesis and vaccine pattern. Development of the viruses that were adapted to tupaia may be a strategy for making this model more durable and effective (6,7,8).

Mouse Models for Hepatitis C Virus

Rodent Models of Hepatitis C Virus Infections

Mice and rats have natural resistance to HCV infection since rodent hepatocytes do not support entry of HCV and replication. Different mouse models can be designed due to genetic structure changes (7,8). Thus, elimination of host factors preventing HCV infection and providing exogenous human factors required for host are possible for success of process. Hence, having information about factors causing human tropism caused by HCV is important (9).

A large number of studies have been carried out for understanding why mouse hepatic cells were naturally resistant to entry of HCV and why ineffective replication was allowed in cell cultures and animals. It has been shown that mouse hepatocytes permitted entry of HCV in cell cultures containing genetically modified cells on the purpose of expressions of HCV specific entry factors CD81 and occludin (OCLN) found in human (2).

Several genetically modified mice (i.e. transgenic mice) that carry different components of the HCV genome had been the first mouse models for the authors to examine the HCV-host interaction. These transgenic mice show liver pathologies that are mimicking humane diseases, primarily steatosis and primary liver cancer. However, contrary to those in human beings, the immune system of the mice has a tolerance for viral proteins that are transgenically expressed, and liver pathogenesis develops if there is no local inflammation. In addition, in these mice, the lack of active HCV RNA replication makes the study of HCV infection impossible. Therefore, subsequent studies focused on developing rodent models which supported productive HCV infection by humanizing mice or rats to make them permit HCV (7). As a result, if this resistance can be overcome, a valuable mouse model can be obtained and the improvement of potential immune strategies that will block the entry and replication of HCV in human beings can be provided (2).

In human liver-chimeric mouse models, generation of proliferative signal for human hepatocytes is provided by inflicting damage on mouse hepatocytes. For the creation of injury in the mouse liver, four models have been described. The first and most widely-used model is based on transgenic over-expression of urokinase-type plasminogen activator (uPA) in the mouse liver (8). The uPA-severe combined immunodeficieny (SCID) mouse model, in which the liver of these chimeric mice can be nearly completely repopulated by the transplanted human hepatocytes, was for first described 15 years ago (7). The improvement of liver dysfunction was determined in mice overexpressed the uPA under the albumin promoter control. However, hepatocytes selectively proliferated and, then, repopulated slowly whole liver in mice in which transgene underwent somatic deletion. In case of suppression of immune system in these mice, vaccination of hepatocytes belonging to different species was possible. However, high level humanization of mouse liver is possible with transplantation of finest quality hepatocytes to homozygous mice in terms of uPA-transgene (9).

Researchers have developed uPA mouse model with SCID (18,19). Due to the overexpression of the uPA transgene in their liver, these immunodeficient (SCID) mice with hepatocyte-lethal phenotype can efficiently be engrafted with primary human hepatocytes in order to initiate HCV infection (7). This immunocompromised model has been shown to support the reproduction of transplanted human hepatocytes. More important than this, it was determined that HCV infection continued by detecting viral RNA in hepatocytes after IV inoculation (2). It has been reported that after a viral inoculation, the HCV titers of over 10^7 IU/mL could be observed and viral infection could be sustained up to 10 months (18,20,21). Studies have shown that when human hepatocytes constituted the majority of the liver cells in SCID mice (min. 80% out of the complete hepatocytes), infection with HCV carried out and this infection could cause a liver failure (2). It is important that these rats may be infected with all the genotypes of patient-based viruses in addition to JFH1-based viruses just as it is the case in chimpanzees. This facilitates the study of various different inocula by authors (9).

The uPA-SCID model has been widely utilized for the purpose of evaluating the strategies for preventing or treating HCV infection. One of these approaches is targeting the cell entry of the virus. HCV entry is a very important step to establish infection and can be

blocked by using neutralizing antibodies which bind to the virions or by monoclonal antibodies (mAbs) which target the host entry factors (Table 1) (7).

It has been shown that usage of neutralizing antibodies that are purified from blood of a genotype 1a-infected patient was effective to inhibit viral infection with homologous and heterologous HCV strains. In addition to antibodies that target the virus, HCV entry could also be inhibited by targeting host factors required for this entry (22). Studies have shown that apolipoprotein (ApoB) and cholesterol ester transfer protein can play a role in allowing HCV infection in SCID mice and this can be targeted for prevention of viral entry in humans (23).

It has been reported in studied with different genotypes that mAbs against the HCV entry factors CD81 and SR-BI have been tested successfully in the uPA-SCID mouse model and mAbs were effective for inhibiting HCV infection (24,25). In addition, within 3 days after injection, chimeric-liver mice were shown to be able to be treated with five injections of 400 µg of anti-SR-BI mAb (25). Also, the uPA-SCID model was utilized successfully to assess the efficiency of drugs with small-molecules and various other molecules that target the host entry factors. Moreover, the uPA-SCID mouse model was utilized to evaluate the efficiency of recently developed direct acting antivirals (DAAs) specifically targeting proteins encoded by HCV and required for viral replication (7). Likewise, HCV-infected SCID mice were utilized to investigate the efficacy of DAAs with IFN alpha-2, anti-NS3 and anti-NS5B proteases and received responses were shown to be parallel to those in humans beings. Hence, studies conducted with SCID mice have a potential bridge between *in vitro* and clinic studies for HCV antiviral agents (2).

Although the uPA-SCID mouse model has proven its beneficial sides and has become relevant for the preclinical assessments of new antiviral compounds, it has some limitations as well. These mice that are very fragile have to be engrafted in the first weeks of their lives providing that they are born with a hepatocyte-lethal phenotype. In addition, the uPA transgene can be deleted in some mice. This leads to the restoration of a wild-type phenotype, and then, to the loss of the human hepatocyte graft. Another disadvantage of this model is that there is no functional adaptive immune systems. Because of the serious combined immunodeficiency, these mice do not have functional mature T and B cells. For this reason, they cannot be utilized to study adaptive immune responses or for the assessment of vaccines. However, these mice do not lack innate immunity and were used to investigate HCV-specific innate immune responses (7).

The lack of complex immune system and completed humanized liver are among limiting factors in SCID mouse model. Recent studies are hopeful with using herpes simplex type 1 kinase/ganciclovir system for cell-specific ablation on the purpose of production of completed human hepatocytes in SCID mice (2).

For the purpose of overcoming the difficulties of steady generation of human liver mice, another model has been developed by using genetically modified immune-deficient mice leading to hepatocyte lethal phenotype. When FAH_/_ animals were crossed with inactivated recombination activating 2 (RAG2_/_) and IL-2 receptor c-chain (IL-2Rcnull) mice, extensive humanization of liver was possible and this model was called as FRG (FAH_/_ RAG2_/_ IL2rg_/_) model (7,9).

These "FRG" mice lack the Rag2 recombinase and the common γ -chain of the interleukin receptors, and this leads to a more profound immunodeficiency. Moreover, these mice do not have enzyme fumarylacetoacetate hydrolase (Fah) having a catabolic role in tyrosine, which leads to liver degeneration (7). In this case, accumulation of fumarylacetoacetate and succinylacetone, which are the toxic metabolites of liver, occurs by forming failure in tyrosine catabolic pathway (9). In this model, it is possible to prevent the liver degeneration by administering the 2-(2-nitro-4-trifluoromethylbenzoyl)- 1,3-cyclohexanedione (NTBC) medication to the mice. By doing so the time of the transplantation of human hepatocytes becomes easier to be controlled than in the uPA-SCID mouse model because hepatocellular damage in liver occurs at any time in a serious spirit with cessation of drug. In addition to this, contrary to the uPA-SCID mouse model, spontaneous reversion of the hepatocyte-lethal phenotype does not occur due to the full deletion in the Fah-encoding gene (8).

Human hepatocytes may be efficiently transplanted to FRG mice for the purpose of obtainingliver repopulation by terminating the usage of NTBC. Up to now, in the FRG model, it has been reported in only one study that transplanted mice were successfully infected with a genotype 2a HCV JFH-1 strain, a clinical isolate of HCV genotype 1a and chimeric genotype 1a/2a and 1b/2a viruses (26). So far, this chimeric liver mouse model has been less extensively used than the uPA-SCID model due to efficacy of allowing preclinical evaluation of antiviral compounds. Nevertheless, several reports using this model are expected within the next years (Table 1) (7).

Like the uPA-SCID mouse model, the FRG model has an immune system deficiency. Due to the Rag2 and γ -c deficiencies, these mice do not host T-, B- and NK-cells. Thus, these animals are not more suitable than the uPA-SCID mice for the study of HCV immunopathogenesis and for development and assessment of vaccines (7).

Recently, in order to overcome the lack of specific immunity in uPA-SCID and FRG models and in order to resolve incompatibilities between the immune system of rats with human hepatocytes, both human immune cells and human hepatocytes were transferred to rats by combining the adult human hepatocytes and human CD34 + hematopoietic stem cells (HSC) from different human transmitters (9).

Washburn et al. (27) obtained the dual-speed transmission AFC8 HSC/Hep (human HSCs/hepatocyte progenitor) model after intrahepatic injection of CD34 + HSCs and human hepatoblastoma isolated from a single donor. This model is genetically modified to express albumin promoter under the control of high level of caspase-8 and FK506 binding protein (FKBP) in rat liver, it was formed with host apoptotic elimination of hepatocytes in Balb/c Rag2-/- γ -c-/- (BRG) type of rats that have immune deficiency. AP20187 injection stimulates homodimerization of caspase-8 active domains and this situation causes death of rat hepatocytes by eliminating the caspase-8 enzyme. This induced liver deficiency improves the hybridization of human hepatocytes. These rats that were transgenic should be transplanted within the first 5 days of their lives with CD34 + hematopoietic stem cells that obtained from the same fetal liver

and allow compliance human hepatocyte precursor cells and human leukocyte antigen (HLA) hematopoietic stem cells. As a result, these humanized rats have been shown to have human hepatocytes and T-cells. Following infection with HCV, human T-cell immune response to HCV has been observed with the development of inflammation and fibrosis in the liver of the rats. In this model, HCV RNA could be determined only in the liver extracts and was not observed in the plasma of the inoculated rats (2,7,9).

Although there is no viremia, in HCV-specific CD4 and CD8 T cell responses and HCV-positive mouse live, human immune cell infiltration was observed but specific B cell responses were not determined. The absence of functional B cells and absence of serum HCV particles is a serious obstacle to the use of this model in the evaluation of antiviral drugs and potential vaccines (Table 1) (7).

Another model of immunocompetent rat which allow HCV infection, developed immediately after AFC8-huHSC/Hep. In order to study viral entry and immunity, Dorner et al. (28) have generated a humanized rat model by using genetic engineering. Unlike the previous models, rats had a functional immune and live system in this model.

In this model, in order to eliminate the virus cell-type-specific restrictions in entry and to make the rat liver can be infected with HCV, rat hepatocytes are genetically modified with *in vivo* adenoviral vector to express HCV entry factor of human CD81, SR-BI, CLDN1, and OCLN (7,8). By using this model, human-specific CD81 and occludin are essential for all HCV entries in the rat hepatocytes and when combined with CD81 and occludin SCARB-I expression increased HCV entry was determined (2). For this reason, HCV has been granted access to these cell, evaluating the entry inhibitors and vaccination candidates became possible (9).

Since an immunocompetent rat is used in this model, the continuation of viral replication and infection remains limited (2). Therefore, this model remains inadequate for the assessment of DAAs or antivirals which target the collection and stages of the viral life cycle. Also, in order to include human input factor of rat hepatocytes, the use of adenoviral vector and the stimulation of an immune response against vector are a complicated operation related with HCV-specific immune responses. Because, stimulation of the interferon stimulating genes (ISGs) and the fast natural immunity caused by NK cells lead to eventual loss of the hepatocytes to which information was transferred with adenovirus. For this reason, HCV-mediated immunopathogenesis study is not possible in this model. However, this model allows understanding of all immune responses induced by viruses; it is the only MHC rat model which processed in a magnificent way in infected hepatocytes and immune system (7). In order to hinder acute infection before and after the exposure to HCV, passive immunization or vaccination strategies can be used in future research (2).

Rat Model for Hepatitis C Virus Infection

The fact that human-liver chimeric mouse models are immunocompromised models, to prevent rejection of human hepatocytes is another important shortcoming restricting studies regarding immune responses. HCV immunopathogenesis in rats, primary (human) adaptive immune response and vaccine efficacy studies in rats require both a human liver graft and functional (human) immune system in one and the same recipient animal (9).

In order to overcome these obstacles, researches tried to develop a human hepatocytes tolerant and immunocompetent rat model susceptible to HCV infection. Therefore, in order to establish specific tolerance towards human hepatocytes, primary Huh7 human hepatoma cells were injected into the peritoneal cavities of fetal rats between 15-17th days of pregnancy. Within 24 hours after birth, transplantation of Huh7 cells was performed into newborn rats with intrasplenic injection route and it was found that the cells lived without rejection and growth in a limited proportion was observed. In tolerating transplanted rats, transplanted human Huh7 cells that actively synthesize and human albumin in serum and human hepatic mRNA in the liver were determined (29).

The transient viremia was detected as $7x10^3$ copies/ml at 4th week and $2x10^4$ copies/mL by obtained peak at 12th week from these rats after 1 week from transplantation when inoculated HCV from obtained serum. The serum alanine aminotransferase levels, which are biochemical indicators of inflammation in the liver, started to rise at the 4th week and made a peak at the 13th week and then decreased. In periods when there is detectable viremia, mononuclear infiltrates were observed in portal and central areas in liver sections in the examination performed with light microscope (2,7,9).

Lacking the transplanted cells in the primary human hepatocytes, existing the cell line for human hepatoma, transferring a small number of human hepatocytes and low levels (22,500 copy/mL) of viremia when compared with infection in humans are the restriction of this model (2). Although this animal model is immunocompetent and can be infected with HIV, the mismatch between human HLA and rat MHC prevents the study of the adaptive immune response against infected cells hematoma (7,9). However, this model brings with it a rodent model with sufficient size to tolerate the repetitive blood and tissue sampling for studying immune-mediated damage hepatitis and viral entry and replication; also it creates a screening tool for the evaluation of new antiviral agents (2).

Conclusion and Expectations

HCV infection, which can cause liver failure and hepatocellular carcinoma, is a global health issue. Although studies of HCV have been limited due to the restrictions in availability of proper and reliable animal models, a great deal of what we know today related with viral life cycle and host immune responses have been obtained using the existing models. From determination of species-specific HCV receptors (CD81 and OCLN) to the importance of innate immunity that controls HCV replication, there has been a great progress in *in vivo* HCV research. While animal studies had been initially carried out on chimpanzees, with better understanding of the key factors causing the narrow host range of HCV, live animal models like tupaias, mice and rats are currently available. Although these models present opportunities for research on immunological mechanisms, vaccine candidates and treatment, there is a need for further progress in this field. Advances in human liver chimeric mice models have enabled us to have control over the time of induction of liver damage in mice (26). Furthermore, human immune system cells as well as human liver cells could be transplanted to mice (27). For the first time, combination of human liver and immune system has allowed reproduction of complete HCV life cycle in human hepatocytes in the presence

of human immune system, and has enabled research on human adaptive immune responses and HCV liver disease in murine environment. Future improvements in these models are required in order to enhance the level of liver engraftment, to overcome the restrictions in the development of human leukocytes, particularly non-lymphocytic sub-populations, and to be able to use them in vaccine candidate studies. Additionally, models that include Kupffer cells, stellate cells or liver sinusoidal endothelial cells need to be developed to allow investigation of the effects of non-parenchymal cells apart from the hepatocytes in human liver-chimeric mice on pathogenesis of HCV.

In conclusion, despite the utilization of these models, there are important difficulties in development of DAAs that provide more than 90% of sustainable viral response. Inadequacy in specifying an optimum drug level in a personalized treatment regimen, using HCV obtained from a patient, will lead to formation of resistant mutations. Advances in stem cell and tissue engineering as well as improvements in animal models, molecular and cellular approaches would aid in better understanding of viral pathogenesis and development of new drugs and treatment protocols.

Authorship Contributions

Concept: Mustafa Altındiş, Design: Ferhat Gürkan Aslan, Mustafa Altındiş, Data Collection or Processing: Ferhat Gürkan Aslan, Mustafa Altındiş, Analysis or Interpretation: Ferhat Gürkan Aslan, Mustafa Altındiş, Literature Search: Ferhat Gürkan Aslan, Mustafa Altındiş, Writing: Ferhat Gürkan Aslan, Peer-review: Externally peer-reviewed, Conflict of Interest: No conflict of interest was declared by the authors, Financial Disclosure: The authors declared that this study has received no financial support.

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