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TOPIC HIGHLIGHT

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New animal models for hepatitis C viral infection and pathogenesis studies

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Abstract

Hepatitis C virus (HCV) is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). In man, the pathobiological changes associated with HCV infection have been attributed to both the immune system and direct viral cytopathic effects. Until now, the lack of simple culture systems to infect and propagate the virus has hampered progress in understanding the viral life cycle and pathogenesis of HCV infection, including the molecular mechanisms implicated in HCV-induced HCC. This clearly demonstrates the need to develop small animal models for the study of HCV-associated pathogenesis. This review describes and discusses the development of new HCV animal models to study viral infection and investigate the direct effects of viral protein expression on liver disease.

Key words: Hepatitis C virus; Viral infection; Transgenic mice; Pathogenesis

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INTRODUCTION

Despite the fact that infectious diseases account for at least one-third of all deaths worldwide, our capacity to study both their pathophysiology and host immune responses in man is often limited by the lack of simple laboratory models of infection. During the past decade, laboratory animals have been used as models of human diseases. In particular, transgenic mice models have been helpful to the understanding of the molecular basis of human diseases. The story of hepatitis C virus (HCV) started in the 1970s with the emergence of patients suffering from hepatitis syndrome which was not associated to hepatitis A or B infection (HAV or HBV). This new agent was named NANBH, for non-A non-B hepatitis[1]. In 1978, Alter *et al*[2] demonstrated that the inoculation of NANBH human sera in chimpanzees induced liver disease, and in 1989 Choo *et al*[3] identified a third viral agent for hepatitis by cloning a non-simian cDNA from the serum of a NANBH-infected chimpanzee. HCV infection leads to liver cirrhosis in up to 35% of cases and hepatocellular carcinoma develops in 2%-7% of cirrhotic patients per year. It is now accepted that the pathobiological changes induced by HCV infection are due to both the immune response and the direct viral cytopathic effects of the virus. As described by other authors in this issue, there is no prophylactic vaccine against HCV at present, and the therapeutic options are mainly limited by a lack of effective long-term treatment. Like other human hepatitis viruses, HCV needs fully functional human hepatocytes for its development. The discovery of anti-infectious agents and immune defense mechanisms has to date been severely restricted by the ethical and practical constraints of access to receptive cells. Due to the nearly strict human tropism of HCV, only man and higher primates such as chimpanzees have until recently been receptive to HCV infection and development. The purpose of this review is to focus on new HCV animal models independent of the chimpanzee, which will enable the study of viral infection and the direct effects of viral protein expression on liver disease.

NEW ANIMAL MODELS FOR HCV INFECTION

As summarized in Table 1, except for the chimpanzee, the only non-human primate permissive to HCV infection is the marmoset. Tupaia, a member of the Tree shrew genus is equally infected by HCV. The capacity for GBV-B, a hepatotrope virus of the flaviviridae family, to infect tamarins and marmosets has been used as a tool to better characterize HCV virus replication and to test HCV antiviral drugs[4-6]. However, none of these models is

Table 1 HCV infection trials in animals

Virus	Animal tested		Infection	Original reference
HCV	Non human primates	Chimpanzee	Yes	Alter *et al*[2] 1978
		Marmoset	Yes	Feinstone *et al*[71] 1981
		Cottontop tamarin	No	Garson *et al*[72] 1997
		Cynomoglus monkey	No	
		Rhesus monkey	No	
		Green monkey	No	
		Japanese monkey	No	Abe *et al*[73] 1993
		Doguera baboon	No	
		Chacma baboon	No	Sithebe *et al*[74] 2002
	Scandentia	Tupaia	Yes	Xie *et al*[75] 1998
	Rodents	uPA/SCID mice	Yes	Mercer *et al*[26] 2001
		Trimera mice	Yes	Galun *et al*[10] 1995
		Rat	Yes	Wu *et al*[8] 2005
		Woodchuck	No	Abe *et al*[73] 1993

Table 2 Characteristics of HCV infection in three rodent models

Rodent models	Humanization	Viremia	Duration of viral infection	Comments	Publications
uPA/SCID mice	Human hepatocyte transplantation	1×10^4 to 8×10^7 copies/mL	Up to 9 mo with maximum viremia as from one month	High viremia Variability of primary human hepatocytes Immunosuppressed mice	Mercer *et al*[26] 2001 Meuleman *et al*[22] 2005 Kneteman *et al*[29] 2006
Trimera mice	Xenograft of human liver tissue	Around 7×10^4 copies/mL	Around one month with peak viremia at 18 d	Low viremia Variability of human liver tissues Immunosuppressed mice	Ilan *et al*[12] 2002 Eren *et al*[11] 2006
Rat	Immunotolerization and transplantation of a human hepatoma cell line	1-2 × 10^4 copies/mL	Minimum 4 mo with peak viremia at 3 mo	Low viremia Transplantation of a hepatoma cell line Immunocompetent rat	Wu *et al*[8] 2005

perfect, particularly because of their inability to produce numerous animals in a short time (long gestation periods) and their high breeding costs. Rodents are certainly the most appropriate model for all biological studies. Their short gestation period (around 20 d for mice and rats), their small size and their low cost are particularly advantageous. Three interesting models for HCV infection: the immunotolerized rat model, the Trimera mouse model and the uPA/SCID model, are being developed (Table 2, Figure 1).

The rat model

Interestingly, Wu *et al* took account of the fact that the rat immune system does not develop until 15-17 d of gestation. They immunotolerized rat embryos to allow the transplantation and maintenance of a human hepatoma cell line (Huh7)[7] which could be infected with HCV[8] (Table 2, Figure 1C). Briefly, fetal rats are tolerized by an intraperitoneal injection of Huh7 cells into pregnant females at the 17th d of gestation. Twenty-four hours after birth, the rats are intrasplenically transplanted with the same hepatoma cell line which will represent around 6% of total hepatocytes after 14 d of development. Nearly 30% of the transplanted human hepatocytes are positive for HCV core protein after inoculation with HCV (genotype 1) positive human serum (Figure 1C). This new animal model is promising but validation remains necessary, for example by using this model to confirm the antiviral effects of drugs used for anti-HCV therapy in humans.

The Trimera mouse model

The Trimera mouse model involves the development of a chimeric mouse with a different source of tissue[9,10]. BNX (beige/nude/X-linked immunodeficient) mice are preconditioned by total body irradiation and reconstituted with SCID mouse bone marrow. These mice tolerate the transplantation of HCV-infected liver fragments from patients with HCV RNA-positive sera, as well as the transplantation of an *ex vivo* HCV-infected liver fragment[10-12]. The liver fragment is transplanted into the ear pinna or under the kidney capsule. In this way, the transplant can be maintained for several weeks and HCV messengers were detected in the serum for up to one month (Table 2, Figure 1B)[10-12]. The model has been validated as a tool for the testing of antiviral components. During a first set of experiments, an inhibitor of the internal ribosomal entry site and an anti-HCV monoclonal antibody were demonstrated to act as potential HCV

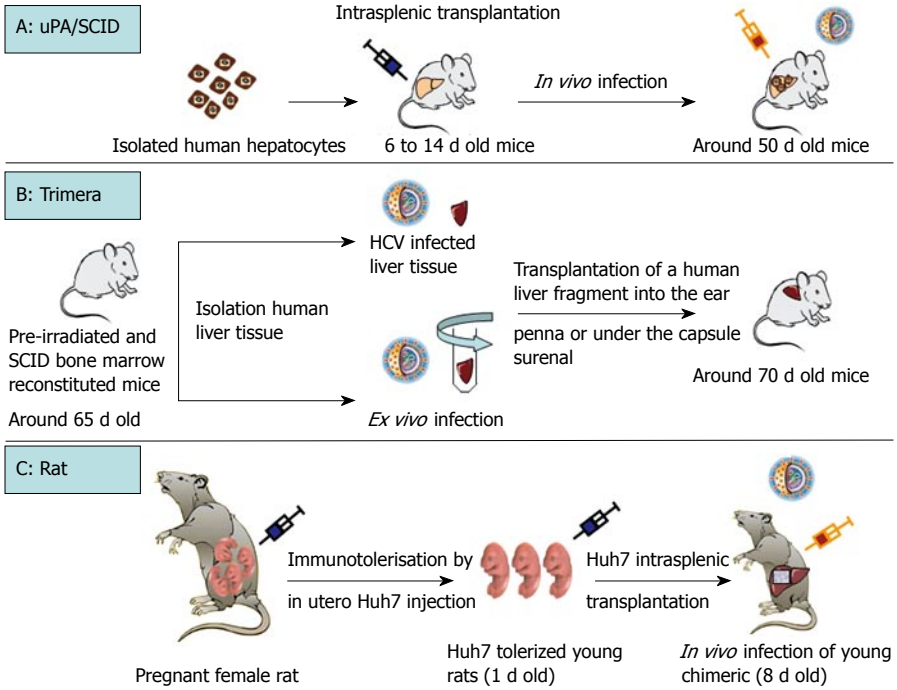

Figure 1 Transplantation protocols of three rodents model of HCV infection. **A**: uPA/SCID model: isolation of human hepatocyte, intrasplenic cell transplantation into homozygous mice and *in vivo* infection by a HCV positive human serum; **B**: Trimera model: irradiation and bone marrow reconstitution of mice, transplantation of intrinsically HCV infected human liver fragment or transplantation of an *ex vivo* pre-infected human liver fragment; **C**: Rat model: immunotolerisation of foetal rats by in utero injection of Huh7 cells, transplantation in new born rats with the same hepatoma cells and *in vivo* infection of young chimeric rats by a HCV positive human serum.

inhibitors[12]. Recently, the same team produced and characterized two monoclonal antibodies directed against HCV envelope protein E2. Following *in vitro* validation of the ability of these antibodies to immunoprecipitate HCV particles, they confirmed an inhibitory effect on HCV infection[11]. Indeed, in the HCV-Trimera model, both monoclonal E2 antibodies were shown to be capable of inhibiting the *ex vivo* HCV infection of human liver fragments (reduction of resultant viremia from 3×10^4 to 3×10^3 copies/mL). A reduction in viremia (3.1×10^4 to 5×10^3 copies/mL) was also demonstrated when these antibodies were used to treat HCV-trimera mice[11]. In conclusion, this mouse model appears to be well-suited to evaluating the inhibitory capacity of drugs, as had previously been shown with monoclonal antibodies directed against HBV[13,14]. Nevertheless, we should not forget that this approach involves the use of heterotopic and xenogenic grafts, so that we can never be entirely sure of the physiological relevance of observations.

The urokinase plasminogen activator protein (uPA) immunodeficient mouse model

Historically, urokinase plasminogen uPA transgenic mice were described in 1990 by Heckel *et al*[15]. The same team then demonstrated the ability of a small number of "normal" hepatocytes to repopulate ad-integrum the liver of transgenic uPA mice[16]. Indeed, the overexpression of uPA protein in hepatocytes is cytotoxic, giving rise to a continuous liver regeneration process. Under these conditions, hepatocytes which lose the transgene by somatic reversion, as well as healthy transplanted hepatocytes, have a strong survival advantage over resident cells[16,17]. Based on this advantage, uPA transgenic mice were back-crossed on an immunodeficient background (SCID or Rag2 mice) to obtain a mouse model which tolerated the xenotransplantation of human, woodchuck and tupaia hepatocytes[17-22]. Optimum liver repopulation requires the intrasplenic transplantation, within one or two weeks of birth, of high quality hepatocytes into mice which are homozygous for both the SCID trait and uPA transgene (Table 2, Figure 1A). The morphological and biochemical characterization of chimeric mice revealed satisfactory hepatic architecture and fusion of the mouse and human structures, indicating a physiological integration of transplanted cells[20,22]. The functionality of transplanted hepatocytes was attested by their susceptibility to infection with human hepatotropic pathogens such as *Plasmodium falciparum*[23], hepatitis B virus[22,24,25] and hepatitis C virus[22,26-28]. It was subsequently shown that this HCV-infected humanized mouse could be used to demonstrate the antiviral activity of two compounds which had already been shown to be effective during clinical trials: the administration in mice of IFNα2b and an anti protease agent (BILN-2061) significantly reduced HCV viremia[29]. Furthermore, it was shown that the antiviral effect was dependent on the viral genotype but appeared to be independent of the provenance of human hepatocytes. Interestingly, another team confirmed the antiviral effect of BILN-2061, but they also encountered cardiotoxic adverse effects with this compound (unpublished data, ISVHLD 2006 congress, Vanwolleghem *et al*). The uPA/SCID model is therefore appropriate for testing new antiviral molecules by evaluating their efficacy and toxicity.

It is clear that the immune response to viral infection plays a major role in the outcome of liver disease. By taking advantage of the absence of adaptive immune response in the chimeric uPA/SCID mouse model, Walters *et al*[30] were able to investigate the role of the innate antiviral immune response to HCV infection. The purpose of their study was to distinguish virus-induced gene expression changes from adaptive HCV-specific immune-mediated effects. Globally, in the uPA/SCID mouse model, HCV infection activates the transcription of interferon-stimulated genes which are in particular implicated in establishing the

innate immune response, and thus active in the inhibition of HCV replication. As previously shown in HCV-infected patients and HCV transgenic mice, these authors confirmed in the uPA/SCID mouse model the relationship between a severe HCV infection and lipid metabolism perturbation, suggesting that liver disease may not be mediated exclusively by an HCV-specific adaptive immune response. Thus, the innate immune response may play a fundamental role in limiting the viral HCV RNA copy number and can thus slow the progression of infection.

In summary, the recent development of small animal models for experimental HCV infection has opened new perspectives for the evaluation of novel therapeutic and/or prophylactic compounds against HCV. Indeed, these three rodent models are really promising, although relatively complicated to use, but they present the unquestionable advantage of being much less expensive and easier to maintain and breed than primates. The rat model may be the most accessible, notably because of the immunocompetent nature of the animals and the larger number of reproducible infected animals that could be obtained in theory using hepatocyte cell line transplantation. The two mice models are more physiologically relevant, in that they are based on the transplantation of human tissue or primary hepatocytes. The model most closely related physiologically to humans is certainly the uPA/SCID mouse. Indeed, even if this model is developed in an immunotolerant setting, humanized liver may contain around 75% of human hepatocytes as compared to just 6% of hepatoma cells in the rat (Table 2). Furthermore, viremia clearly lasts longer and at higher levels in uPA/SCID mice than in other models (Table 2). Under these conditions, the uPA/SCID mouse model appears to be the most relevant to building a bridge between *in vitro* research and clinical trials.

HCV TRANSGENIC MOUSE MODELS

An increasing body of evidence suggests a direct involvement of HCV in cellular metabolic disturbances. As described in this report, conditional expression of the HCV genome in transgenic mice has enabled study of the direct effect of HCV on hepatocytes, and investigation of the molecular pathways of HCV associated with liver injury. This has generated data crucial to our understanding of several aspects of HCV pathogenesis. As reported in Table 3, and despite some contradictory results, the development of HCV transgenic mouse models has enabled evaluation of the different, direct cytopathic effects of HCV protein and their correlation with the pathogenesis of chronic hepatitis C.

Hepatic steatosis and the derangement of lipid metabolism are characteristic of HCV protein expression

Recently, a correlation between HCV infection and both diabetes and insulin resistance was suggested, indicating that they might be metabolic diseases associated with viral infection[31,32]; this could be a critical factor in the pathogenesis of chronic hepatitis C. Transgenic mouse models have demonstrated a link between HCV core protein expression and elevated serum insulin levels, associated with a minor elevation of plasmatic glucose but without the development of diabetes[33,34]. Furthermore, the administration of insulin resulted in higher glycemia when compared to values in non-transgenic mice, indicating the presence of an insulin resistance phenotype in core transgenic mice[33,34]. This insulin resistance may be due to down-regulation in the expression of insulin receptor substrates 1 and 2 (IRS1 and 2), probably *via* an up-regulation of the suppressor of cytokine signaling 3 (SOCS3)[35]. One possible mechanism is that HCV core-induced SOCS3 promotes the proteosomal degradation of IRS1 and IRS2 through ubiquitination[35]. These results therefore provide direct experimental evidence for a role of HCV core protein in the development of insulin resistance mechanisms in HCV-infected patients.

Hepatic steatosis, which involves an accumulation of intracytoplasmic lipid droplets, is a common histological feature which is observed in more than 50% of chronic hepatitis C carriers[36]. Both host and viral factors have been demonstrated to play an important role in its development. By accelerating the development of fibrosis, steatosis may contribute to the progression of liver disease and the development of HCC. Consistent with the implication of viral protein in steatosis, it has been reported that the core protein of HCV targets microsomal triglyceride transfer protein activity, modifies hepatic VLDL assembly and secretion and increases the concentration of monosaturated fatty acids[37,38]. Furthermore, alcohol consumption in core transgenic mice has been shown to increase hepatic lipid peroxidation and hepatic TNF alpha and TGF beta expression[39]. This may participate in activating fibrogenesis and hence the development of HCC observed in HCV patients who abuse alcohol.

HCV and development of hepatocellular carcinoma

Epidemiological evidence favors a direct role for HCV in the development of hepatocellular carcinoma (HCC). Despite some contradictory results, transgenic mouse models have demonstrated the implication of core protein expression in HCC development. Moriya *et al*[40-42] showed that core protein expression in mice led to steatosis, oxidative stress and ultimately HCC in aging mice. RXR alpha is activated by cellular retinol binding protein II (CRBPII) in the liver of core-expressing transgenic mice[43]; suggesting that the modulation of RXR alpha-controlled gene expression *via* its interaction with core protein could contribute to liver pathogenesis. Alterations to other signaling pathways *via* activation of signal transducer and activator of transcription 3 (STAT3), activator protein-1 (AP-1), MAPK and the suppression of SOCS-1 expression may equally contribute to tumorigenesis in HCV core-expressing transgenic mice[44-47]. In another core transgenic line, the development of malignant lymphoma and hepatocellular adenoma has been observed[48]. In other reports, neither steatosis nor hepatic tumors were detected in core transgenic mice[49,50]. However, HCC was more frequently observed after diethylnitrosamine treatment or the repeated administration of carbon tetrachloride[49,51].

Table 3 Transgenic mice expressing HCV proteins

Viral protein	Original reference of the transgenic mouse	Mouse strain	Promoter	Comments	References
Core	Moriya *et al*[42] 1997	C57BL/6	HBV	Steatosis	Moriya *et al*[40] 1998
				HCC	Moriya *et al*[42] 1997
				Oxidative stress	Moriya *et al*[41] 2001
				Increased concentration of monounsaturated fatty acids	Moriya *et al*[37] 2001
				Inhibition of microsomal triglyceride transfer protein activity and VLDL secretion.	Perlemuter *et al*[38] 2002
				Alcohol and core protein increase lipid peroxidation	Perlemuter *et al*[39] 2003
				Alteration of intrahepatic cytokine expression and AP-1 activation	Tsutsumi *et al*[45] 2002
				Interaction with retinoid X receptor alpha	Tsutsumi *et al*[43] 2002
				Cooperation with ethanol activation of MAPK	Tsutsumi *et al*[44] 2003
				Insulin resistance	Shintani *et al*[34] 2004
					Koike *et al*[33] 2006
				Modulation of interferon pathway (Inhibition of SOCS-1 expression)	Miyoshi *et al*[47] 2005
				ER stress, apoptosis	Benali-Furet *et al*[78] 2005
Core	Honda *et al*[52] 2000	C57BL/6	HBV	Modulated sensitivity to Fas-mediated apoptosis	Honda *et al*[52] 2000
				Insulin resistance via down-regulation hepatic IRS1 and 2	Kawaguchi *et al*[35] 2004
				Constitutive activation of STAT3, implication in HCC	Yoshida *et al*[46] 2002
Core	Pasquinelli *et al*[50] 1997	C57BL/6	Major urinary protein	No liver disease	Pasquinelli *et al*[50] 1997
Core (Korean wt and mutants)	Wang *et al*[79] 2004	C57BL/6J	HBV	Cell dysplasia for S99Q core mutant	Wang *et al*[79] 2004
Core (in T cell)	Soguero *et al*[53] 2002	C57BL/6	CD2	Increased Fas-mediated apoptosis and liver infiltration of peripheral T cells	Soguero *et al*[53] 2002
Double Tg core X TCR	Cruise *et al*[55] 2005	C57BL/6 × DO11.10 (H-2d)		Increased Fas ligand expression of CD4+ T cells associated with liver inflammation	Cruise *et al*[55] 2005
				Role of CXCR3 ligands via Fas induction, during the inflammatory response	Cruise *et al*[54] 2006
Core	Ishikawa *et al*[48] 2003	C57BL/6N	serum amyloid	Malignant lymphoma and Hepatocellular adenoma in old mice	Ishikawa *et al*[48] 2003
Core	Kato *et al*[49] 2003	C57BL/6	serum amyloid P	Adenoma and HCC development in transgenic mice following repeated CCl_4 administrations.	Kato *et al*[49] 2003
Core Core-E1-E2	Kamegaya *et al*[51] 2005	FVB × C57BL/6	Albumin	After DEN treatment, core-E1-E2 mice develop tumors with a larger size than core mice (diminution of apoptotic index)	Kamegaya *et al*[51] 2005
Core-E1-E2-p7	Lerat *et al*[62] 2002	C57BL/6	Albumin	Steatosis	Lerat *et al*[62] 2002
				HCC (rare)	
				Sensitivity to oxidative stress	Okuda *et al*[80] 2002
Core-E1-E2-p7	Korenaga *et al*[81] 2005	C57BL/6J	Albumin	Increase in ROS production by mitochondrial electron transport complex I	Korenaga *et al*[81] 2005
Core-E1-E2	Kawamura *et al*[82] 1997	FVB	Albumin or major urinary protein	No liver disease	Kawamura *et al*[82] 1997
Core-E1-E2	Honda *et al*[56] 1999	C57BL/6	H2-Kd	Sensitivity to anti-fas administration	Honda *et al*[56] 1999
Core-E1-E2	Naas *et al*[57] 2005	C57BL/6	CMV	Steatosis. Acceleration of liver and lymphoid tumor development	Naas *et al*[57] 2005
Core-E1-E2-NS2	Wakita *et al*[59] 1998	Balb/C	Cre/Lox system (CAG promoter)	Hepatitis injury associated with HCV-specific CTL response	Wakita *et al*[59] 1998 Wakita *et al*[58] 2000
				Suppression of Fas-mediated cell death	Machida *et al*[83] 2001
				HCV-specific CD8+ CTLs specifically induce liver injury	Takaku *et al*[62] 2003
E1-E2	Koike *et al*[63] 1995	CD1	HBV	No liver disease Expression in salivary glands	Koike *et al*[63] 1995
				Exocrinopathy resembling Sjogren syndrome	Koike *et al*[84] 1997
E2	Pasquinelli *et al*[50] 1997	C57BL/6	Albumin	No liver disease	Pasquinelli *et al*[50] 1997
NS3-NS4A	Frelin *et al*[64] 2006	C57BL/6	Major urinary protein	No liver disease. Alteration of hepatic immune cell subsets. Reduced sensitivity to TNF alpha mediated liver disease.	Frelin *et al*[64] 2006

NS5	Majumder *et al*[65] 2002	FVB	ApoE	No liver disease	Majumder *et al*[65] 2002
				Protection against TNF alpha mediated liver disease (inhibition of NF-kappaB activation)	Majumder *et al*[66] 2003
Full polyprotein	Alonzi *et al*[60] 2004 Blindenbacher *et al*[67] 2003	C57BL/6	Alpha1 antitrypsin	Steatosis T cell infiltrate	Alonzi *et al*[60] 2004
				Inhibition of IFN alpha-induced signaling	Blindenbacher *et al*[67] 2003
				Increased expression of protein phosphatase 2A	Duong *et al*[68] 2004
Full polyprotein	Lerat *et al*[62] 2002	C57BL/6	Albumin	Hepatic steatosis, HCC	Lerat *et al*[62] 2002
				Impairment of intrahepatic immune response (absence of elimination of adenovirus-infected hepatocytes)	Disson *et al*[85] 2004
				Down-regulation of pro-apoptotic CIDE-B protein in adenovirus-infected transgenic mice	Erdtmann *et al*[86] 2003
				Iron overload induces increase the risk of HCC (mitochondrial injury)	Furutani *et al*[87] 2006

HCV-core protein in liver cells may affect the persistence of Fas-mediated liver cell injury[52]. Liver inflammation induced by HCV core-expression in CD4+ T cells is associated with high expression levels of the Fas ligand and CXCR3 chemokine induction[53-55]. In this context, liver inflammation is abolished by anti-Fas antibody treatment[54,55]. Similarly, transgenic mice expressing Core-E1-E2 present with hepatocyte necrosis associated with increased Fas-mediated injury[56,57]. Furthermore, the expression of core-E1-E2-NS2 viral proteins, as well as of the entire HCV polyprotein, may induce steatosis with lymphocyte cell infiltrate, mito-chondrial injury and sensitivity to oxidative stress and HCC in aging animals[58-62]. By contrast, E1-E2, E2, NS3-NS4A or NS5A transgenic animals have not been shown to exhibit any major histological changes to the liver[50,63-66].

HCV and interferon pathway modulation.

In vivo experiments in transgenic mice have confirmed that the expression of full HCV polyproteins and core protein inhibits interferon alpha signaling. The HCV core protein has been shown to induce an aberrant expression of SOCS1, which can suppress Jak-STAT signaling activation[47]. Similarly, STAT signaling was found to be strongly inhibited in the liver of HCV transgenic mice[67]. In this model, STAT phosphorylation by Jak was not reduced, but the binding of STAT transcription factors to the promoters of interferon-stimulated genes was inhibited[67]. HCV expression in the liver is associated with the inhibition of STAT function by concomitant induction of the expression of the protein inhibitor of activated STAT (PIAS)[68]. This may be mediated *via* an up-regulation of protein phosphatase 2A and by STAT demethylation[68,69]. In addition, NS5 protein activates STAT3 through interaction with Jak1[70].

In summary, and despite some discordant data, studies involving transgenic mice expressing one or a combination of HCV viral proteins have been essential to a clearer understanding of the mechanisms involved in HCV-induced pathogenesis. However, to be rigorous the interpretation of any data must take account of variations in the genetic background of mice, constitutive transgene expression and the absence of any immune response. Nevertheless, even if we consider the variability of morphological observations and the diversity of biochemical data, most reports strongly support a direct role for core protein in liver pathogenesis. Core protein may predispose subjects to HCC development through its contribution to the onset of steatosis, fibrosis and oxidative stress, particularly by acting on the expression of cell growth-related genes, the interferon pathway and lipid metabolism.

CONCLUSION

This review underlines the usefulness of rodent models in the field of HCV infection studies. Indeed, as pointed out, exponential information has been obtained using small animal models which are susceptible to HCV infection or allow HCV protein expression. The principal advance in this area is the establishment of small animal models which can support the entire life cycle of the virus. However, the contribution of different viral proteins to HCV-related pathogenesis is far from being clarified. Indeed, if we are to reach definite conclusions regarding the mechanisms linked to HCV pathogenesis, the next step must be to develop a rodent model which can harbor both a human immune system and human liver cells susceptible to HCV infection. This rodent model will constitute a new tool to allow the efficient screening of HCV vaccine candidates. Finally, without forgetting the indispensable role of the chimpanzee (which remains the optimum model to study the efficacy of future vaccines), new animal models such as transgenic mice and HCV infection-permissive rodents constitute extremely promising and complementary tools which will enable us to better understand and fight against HCV infection.

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