An Experimental Mouse Model for Hepatitis C Virus

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Abstract: Chronic hepatitis C virus (HCV) infection affects approximately 170 million people and is a major global health problem because infected individuals can develop liver cirrhosis and hepatocellular carcinoma. Despite significant improvements in antiviral drugs, only around 50% of treated patients with genotype 1 and 4 demonstrate HCV clearance. Unfortunately, an anti-HCV vaccine is still not available. To progress treatment of HCV, it is necessary to understand the mechanism(s) by which HCV infects hepatocytes, and how the host immune response prevents the spread of the virus. Because HCV infects only humans and chimpanzees, it is difficult to evaluate immune response mechanisms, and the effects of chemicals and new technologies on these response mechanisms. These difficulties underline the importance of establishing a small HCV-infected animal model. This review focuses on the progress made in recent years towards the development of an experimental mouse model for HCV.

Key words: apoptosis, B cell lymphoma, HCV, immune response, transgenic mice

Introduction

Hepatitis C virus (HCV) is a non-cytopathic, hepatotropic member of the Flaviviridae family, causing acute and chronic necroinflammatory liver diseases [25]. Chronic HCV infection has caused an epidemic with approximately 170 million people infected worldwide and three to four million people newly infected each year [25, 35]. Natural history studies show that 5–20% of patients develop cirrhosis after about 20 years of infection [1, 42, 49]. An increasing number of patients with cirrhosis will develop hepatocellular carcinoma. End-stage liver disease due to chronic HCV infection is the leading cause of liver transplantation in the western world [36]. Furthermore, co-infection with HCV and human immunodeficiency virus (HIV) results in more serious liver cirrhosis than HCV infection alone and the mortality of HIV-infected HCV patients is a serious problem in the USA [44, 55].

The HCV genome is a 9.6-kb, uncapped, linear, single-strand RNA molecule with positive polarity that serves as a template for both translation and replication. Translation of the plus-strand RNA initiates at an internal ribosomal entry site, resulting in the production of a single polyprotein precursor that is processed into structural (C, E1, E2, p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) protein subunits by host and viral proteases [8, 9, 37, 46]. Because of the lack of a proofreading func-
tion for the RNA-dependent RNA-polymerase (NS5B), HCV has a high mutation rate and exists as a genetically heterogeneous quasispecies in individual patients [8].

It is well established that the pathogenesis of HCV infection is controlled by host-virus interactions mediated by the immune response [4, 5, 13]. It has been difficult to clarify the relationship between HCV and the host immune response because of the fact that HCV is only infectious in humans and chimpanzees [3, 11]. It has also been demonstrated that viral clearance during self-limited acute HCV infection is characterized by a vigorous, polyclonal CD4 and CD8 T-cell response [6, 12, 29, 38, 43]. However, the lack of activity in inducing an effective antigen-specific CD4 and CD8 T-cell response in chronically infected HCV cases has been studied in chimpanzees [50], and also in humans infected via needle stick accidents [47]. These studies suggest that a poor immunological response to HCV probably leads to persistent infection. Failure to effectively produce antigen-specific T-cells is possibly the result of viral overload [15–17] and the high level of regulatory T cells present [40]. However, it remains unknown why HCV infection causes persistent infection. It has also been demonstrated that viral replication is inhibited by antigen-specific T cells as well as natural killer (NK) cells, natural killer T (NKT) cells and macrophages in the liver of hepatitis B virus (HBV) transgenic mice [18, 21, 22], indicating that viral clearance requires innate and acquired immune responses.

In this review, we describe an experimental mouse model for HCV infection which allows us to analyze the mechanisms of chronic persistent infection.

**HCV Transgenic Mice Models**

*Generation of HCV transgenic mice using the Cre/loxP switching system*

A transgenic mouse model using a stable expression system generates immunotolerance to transgene products. Therefore, an HCV protein switching expression system may be suitable for in vivo assays of HCV protein effects, as HCV is thought to infect humans with a mature immune function [1]. Using the Cre/loxP system, we developed a transgenic mouse model with efficient conditional transgene activation of HCV cDNA (core, E1, E2, and NS2) (Fig. 1), and two transgenic lines, CN2-8 and CN2-29, were generated [48]. The Cre/loxP system has been used in combination with a recombinant adenovirus vector expressing Cre to alter gene expression in the livers of transgenic mice [34]. Temporal control of viral gene expression using a conditional transgene activation system enables detailed analysis of the immune responses in the host, and observation of cytopathic effects due to viral proteins. HCV proteins were mainly detected in the livers of conditionally expressing transgenic mice. Efficient recombination was observed in transgenic mice livers upon intravenous administration of adenoviruses expressing Cre DNA recombinase. After transgene activation, most hepatocytes were stained with anti-core polyclonal antibody, and 21-, 37-, and 64-kDa proteins were detected by western blot analysis in liver lysates using anti-core, -E1, and -E2 monoclonal antibodies, respectively. Serum core protein was detected in transgenic mice 7 days after transgene activation with concurrent increases in serum alanine aminotransferase levels. Importantly, we observed no significant histopathological changes between infected and uninfected CN2 transgenic mice when CD4- and CD8-positive cells were depleted in the infected mice (Fig. 2). These results suggest that HCV structural proteins are not strongly cytopathic to hepatocytes in the absence of an immune response.
**HCV transgenic mice are resistant to Fas antibody-mediated lethality**

The role of apoptosis in HCV infection is not well defined. Furthermore, the kinetics and extent of hepatocyte apoptosis as well as the pro- and anti-apoptotic mechanisms involved remain unclear. We demonstrated that transgene expression in HCV transgenic mice causes resistance to Fas antibody-stimulated lethality [27]. Apoptotic cell death in the livers of HCV protein-expressing mice was significantly reduced compared to non-expressing mice. Histopathological analysis and DNA fragmentation analysis revealed that the HCV proteins suppressed Fas-mediated apoptotic cell death. To identify the target pathway of HCV proteins, we characterized caspase activity and showed that the activation of caspase-9 and caspase-3/7, but not caspase-8, was inhibited by HCV proteins. In addition, cytochrome c release from mitochondria was inhibited in HCV protein-expressing mice. These results indicate that the expression of HCV proteins may have directly or indirectly inhibited Fas-mediated apoptosis and cell death in mice by repressing the release of cytochrome c from mitochondria, thereby suppressing caspase-9 and caspase-3/7 activation. Furthermore, HCV might cause persistent infection as a result of suppression of Fas-mediated cell death and inhibition of HCV-infected hepatocyte rejection in the liver, given that numerous viruses have been reported to evade apoptotic mechanisms resulting persistent infection [23].

**Fig. 2.** Histopathology of livers of HCV Tg mice. A, uninfected CN2-8 mouse. B, CD4+ and CD8+ cell-depleted CN2-8 mouse infected with AxCANCre and analyzed at day 7. C, CN2-8 mouse infected with AxCANCre and analyzed at day 7. (×250 magnification).

**Fig. 3.** Serum ALT level and histological analysis of livers after anti-Fas antibody administration. A, serum ALT levels of anti-Fas antibody-injected HCV non-expressing (open circles) and expressing (closed circle) mice. ALT levels are expressed as the mean ± SD of three individual experiments. B, hematoxylin and eosin staining of liver sections from transgenic mice at pretreatment and 4 h after anti-Fas antibody injection.
The relationship between chronic HCV infection and lymphoma during interferon (IFN) disruption

It has been demonstrated that HCV infection causes lymphoproliferative diseases, such as B cell non-Hodgkin’s lymphomas and mixed cryoglobulinemia [25, 39]. We established IFN regulatory factor-1-null (irf-1–/–) mice with inducible and persistent expression of HCV structural proteins (irf-1/CN2 mice), in order to evaluate the molecular mechanisms of lymphoproliferation associated with the disruption of IFN signaling and chronic HCV infection [28]. Irf-1/CN2 mice had extremely high incidences of lymphomas and lymphoproliferative disorders and displayed increased mortality. Disruption of irf-1 reduced their sensitivity to Fas-induced apoptosis and decreased the levels of caspase-3/7 and caspase-9 mRNA species and associated enzymatic activities. Furthermore, the irf-1/CN2 mice showed decreased activation of caspase-3/7 and caspase-9 and increased levels of interleukin (IL)-2, IL-10, and Bcl-2, which promote oncogenic transformation of lymphocytes. Disruption of IFN signaling resulted in the development of lymphomas, indicating that differential signaling occurs in lymphocytes rather than in the hepatocyte. IRF-1-inducible genes probably play essential roles in suppressing HCV-induced lymphomas and in eliminating HCV protein-expressing cells. Our transgenic mice provide evidence that the overexpression of apoptosis-related proteins, including Bcl-2, and/or aberrant cytokine production are primary events in HCV-induced lymphoproliferation.

HCV proteins expressed in B cells cause lymphoma

To extend the above-mentioned study with regard to the interaction of lymphoma and HCV infection, we established HCV transgenic mice that expressed the full HCV genome in B cells (RzCD19Cre mice) and observed a 25.0% incidence of diffuse large B cell non-Hodgkin’s lymphomas within 600 days after birth [19]. The incidence of B cell lymphoma significantly correlated with the level of soluble IL-2 receptor alpha subunit (sIL-2Ralpha) in RzCD19Cre mouse serum. All RzCD19Cre mice with substantially elevated serum sIL-2Ralpha levels (>1,000 pg/ml) developed B cell lymphomas. Compared with tissues from control animals, the B cell lymphoma tissues of RzCD19Cre mice expressed significantly higher levels of sIL-2Ralpha. We showed that the expression of HCV in B cells promotes non-Hodgkin’s-type diffuse B cell lymphoma, and therefore, the RzCD19Cre mouse is an appropriate model for studying
the mechanisms related to the development of HCV-associated B cell lymphoma [19].

**Chronic persistent expression in HCV transgenic mice**

We generated another switching system to study the expression of HCV proteins using Mx promoter-driven Cre recombinase with poly(I:C) induction. The Mx promoter is active in hepatocytes as well as in hematopoietic cells. We crossed CN2 mice with Mx1-Cre transgenic mice, in which Cre recombinase is expressed by the IFN-inducible Mx1 promoter. Injection of Mx1-Cre/CN2-29 mice with poly(I:C) induces IFN production and the expression of CN2 gene products in hematopoietic cells (mainly in Kupffer cells and lymphocytes), livers, and spleens but not in most other tissues. As illustrated in Fig. 5, the serum alanine aminotransferase (ALT) levels increased, peaking at 24 h after the first poly(I:C) injection. These serum ALT levels then decreased until day 4, when they increased again until day 6, along with HCV core protein levels. Thereafter, HCV core protein was observed consistently for at least 600 days. We also showed that the serum ALT levels gradually increased after day 210 despite no change in the HCV core protein levels. Histological analysis showed that the HCV core protein was expressed in most hepatocytes of transgenic mice which also exhibited lymphocytic infiltration by the core protein (Fig. 5). These observations indicate that the expression of the HCV proteins caused chronic hepatitis in the CN2-29<sup>+/–</sup>/MxCre<sup>+/–</sup> mice because of a weak and persistent immune response. We observed a number of other pathological changes in these mice, including swelling of hepatocytes, abnormal architecture of liver-cell cords, abnormal accumulation of glycogen, steatosis, fibrosis, and hepatocellular carcinoma. We are convinced that HCV transgenic mice are suitable for evaluating the mechanisms of persistent HCV infection and for assisting with the design of HCV vaccines.

**Role of NK cells in the antiviral effect of HCV transgenic mice**

The liver is enriched with NK cells and this intrahepatic population is embedded in the endothelial lining of the liver sinusoids. These NK cells were originally described as ‘pit’ cells [20]. Intrahepatic NK cells may behave differently to NK cells in other areas because of the ‘tolerogenic’ environment of the liver, with murine intrahepatic NK cells known to be hyporesponsive. They are less cytotoxic and have an altered cytokine profile producing lower levels of IFN-γ and greater levels of immunoregulatory cytokines, such as IL-10, than peripheral blood and splenic NK cells [24]. This hyporesponsive state has been described in the early stages of HBV infection and may contribute to the establishment of chronic viral infection [7]. Peripheral blood NK cell frequencies, both the absolute number and the percentage of the total lymphocyte population, are reduced in chronic HCV compared to healthy individuals [30]. In individuals with chronic HCV infection, NK cell frequency increases following successful antiviral therapy, while a reduction in peripheral blood NK cell frequency in individuals with chronic HCV as compared to spontaneous resolvers has also been noted [10]. Thus, NK cells may play key roles in suppressing HCV replication. We actually observed much higher levels of HCV core proteins in Tg mice with a depleted population of NK cells. Furthermore, Cre-mediated genomic DNA recombination efficiency in HCV-Tg mice was strong in NK cell-depleted mice between 0.5 and 1 day compared to untreated mice. These data indicate that NK cells participate in the elimination of core expressing hepatocytes during the innate immune response in the acute phase of HCV infection [41].

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**Chimeric Human Liver Mice Model**

Mercer et al. generated mice with chimeric human livers by transplanting normal human hepatocytes into...
SCID mice carrying a plasminogen activator transgene (Alb-uPA). Homozygosity of Alb-uPA was associated with significantly higher levels of human hepatocyte engraftment, and these mice developed prolonged HCV infections with high viral titers after inoculation with infected human serum [31].

We used the chimeric mice as they were a vast improvement over the originals, which had a high substitution rate of human hepatocytes [45], and examined the inhibitory effect of DEBIO-025, a novel non-immunosuppressive cyclophilin inhibitor derived from cyclosporin A, on naïve HCV genotypes 1a or 1b in vivo [14]. Collectively, this small animal model is useful for assessing the activity of antiviral compounds [33] and for evaluating protection and passive immunization studies of HCV [26, 32], but because they lack an immune system, this model is not suitable for studies of HCV pathogenesis.

A recent study showed that in Fah–/–Rag2–/–Il2rg–/– mice, the selection pressure for transplanted human hepatocytes can be regulated by the drug 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione. In the absence of this drug, mouse hepatocytes die because of the accumulation of toxic tyrosine catabolites and a lack of fumarylacetoacetate hydrolase, while human hepatocytes remain healthy. These mice have a high level of human liver chimerism, they propagate both HBV and HCV, and the HCV-infected mice are responsive to antiviral treatment. It seems that this human liver chimeric mouse model will be useful for studying HBV and HCV infection, and it has already proven valuable in antiviral drug testing [2].

The development of molecular biological techniques has allowed us to generate transgenic mice. Using these techniques we are able to analyze the immune responses to various viral proteins in mice, even though the virus does not normally infect murine species. It is essential to generate an infectious HCV mouse model for a more precise analysis of the interaction between host and virus. The chimeric human liver mouse model would appear to be a powerful tool for evaluating the effects of antiviral drugs. It is hoped that an experimental mouse model for HCV will yield a number of useful insights into the immunopathogenesis of this viral infection, and assist in the development of antiviral drugs.

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