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News and Commentary

Transgenic models for Hepatitis C virus pathogenesis

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The use of reverse genetics in the mouse, allowing flexible gene manipulation, has been one of the most powerful tools in the last decade for the understanding of complex biological phenomena. Transgenic and knock-out animal models have yet been fruitfully used in the understanding of the molecular basis of several human diseases.[1–3]

Notably, the pathobiology of human viruses has been productively studied in murine models even in the lack of infectivity. One paradigmatic example is Hepatitis B Virus transgenic models which have been instrumental in clarifying many steps of its life cycle and pathogenesis of related disease.[4] A more complex scenario emerges from Hepatitis C Virus (HCV) transgenic mouse models which do not allow as yet unequivocal conclusions.[5,6] In man, pathological changes associated with chronic HCV infection have been ascribed both to the immune response against the virus and to direct viral cytopathic effects.[7,8] The relative contribution of these factors in the onset of the various HCV-related pathological phenotypes, such as liver steatosis, fibrosis, cirrhosis, intrahepatic lymphocyte infiltration and hepatocellular carcinoma, have been explored by transgenic approaches. In the majority of the HCV transgenic models developed so far, the expression of viral protein(s) does not challenge the immune system, because they are recognized as 'self', as confirmed by the lack of anti-HCV antibody production in the transgenic mice.

Has a direct cytotoxic effect been ascribed to specific viral product(s)? The rather contradictory results reported in the literature are summarized in Table 1 where the HCV transgenic mice, that have been developed so far, are listed along with their relative phenotypes, the promoters, the animal strains and the viral protein detection systems used by the various authors. The phenotypic changes described are dissimilar even when the sole core protein is taken in account. Moriya *et al*.[9–11] have shown that expression of the core protein, which was found localized into lipid droplets, nucleus and mitochondria, leads to extensive steatosis, increased oxidative stress, mitochondrial injury and ultimately hepatocarcinoma in aging mice. In a similar transgenic line, reported by Honda *et al*.[12] a major consequence of core expression was a modification in the Fas-induced apoptotic response, but no liver morphological alterations in untreated animals were described. Finally, no liver pathology was observed in the HCV core transgenic mice reported by Pasquinelli *et al*.[13] It is worth noting that similar contradictory results, in terms of modulation of cellular proliferation, transformation and have also been reported in cell culture system apoptotis.[14]

Transgenic animals carrying HCV sequences coding for other structural proteins were also generated. No liver damage was described in the case of transgenic mice expressing the envelope proteins, either E2 alone by Pasquinelli *et al*.[13] or E1 and E2 by Koike *et al*.[15] Interestingly, expression of E1 and E2 proteins driven by a HBV regulatory region, leads to extrahepatic expression which results in exocrinopathy involving the salivary and lachrymal glands, with lymphocyte infiltration and accumulation of fibrous tissues.[16] This pathology resembles Sjogren's syndrome which has been found to be associated with chronic HCV infection in humans.

Expression of the structural proteins core, E1 and E2 driven by an ubiquitous promoter, reported by Honda *et al*.[17] leads to necrosis of hepatocytes that are surrounded by infiltrating lymphocytes. Moreover, these mice show an increased susceptibility to Fas-mediated injury. No liver pathological changes were observed in a similar transgenic line reported by Kawamura *et al*.[18] who, however, limited their observations to 6-month-old animals.[18] A recent report, by Lerat *et al*.[19] showed that liver-specific expression of all HCV structural proteins (core, E1, E2 and p7) led, in aging mice, to steatosis, mitochondrial injury, increased sensitivity to oxidative stress and, at very low frequency, hepato-carcinoma (one mouse out of 42).[20] In the same article the authors described different transgenic lines which express the entire HCV polyprotein.[19] Although protein expression was not detectable in these mice, they presented extensive steatosis, tumor-associated fibrosis and higher frequency of hepato-carcinoma (five out of 37), suggesting that the viral non-structural proteins could contribute to tumorigenesis.

Kohara and coworkers attempted to take into account the contribution of the immunological response in HCV-related pathology, mimicking an acute infection by means of inducible transgenic mice.[21–23] Based on the Cre/loxP technology, they generated a transgenic mouse carrying the core-NS2 cDNA, whose expression is induced by infection with a Cre recombinase-expressing Adenovirus.[21] The authors reported that HCV protein expression resulted in specific anti-HCV cytotoxic T-cell activities and development of a substantial hepatic pathology with increased levels of Alanine Aminotranferase (ALT).[21,22] This liver injury appears to be mediated by T-cells because depletion of CD4+ and CD8+ lymphocytes protects the mice from hepatic damage.[21] Contrary to chronic expression of HCV proteins, which was reported to lead to increased Fas-mediated responses, a significant inhibition of Fas-induced cell death was observed after transient HCV expression, apparently correlating with a block in cytochrome *c* release

Table 1

Viral proteins	Phenotype	Promoter	Protein(s) detection	Mouse strain	References
Core	Steatosis, Carcinoma, Oxidative stress	Hepatitis B virus	(+) Western Blot (+) Immunohistochemistry (+) Electron microscopy	C57BL/6	Moriya *et al.*[9,10,11]
Core	Fas-induced apoptosis increase	Hepatitis B virus	(+) Western Blot	C57BL/6	Honda *et al.*[12]
Core	None	Major Urinary Protein	(+) Western Blot	C57BL/6 × SJL	Pasquinelli *et al.*[13]
E2	None	Albumin	(+) Western Blot	C57BL/6 × SJL	Pasquinelli *et al.*[13]
E1-E2	Exocrinopathy	Hepatitis B virus	(+) Western Blot (+) Immunohistochemistry	CD1	Koike *et al.*[15,16]
Core E1-E2	Necrosis, Lymphocyte infiltration, Fas-induced apoptosis increase	H2K(MHC I)	(+) Immunohistochemistry	C57BL/6	Honda *et al.*[12]
Core E1-E2	None	Albumin Major Urinary Protein	(+) Western Blot (+) Immunohistochemistry	FVB	Kawamura *et al.*[18]
Core E1-E2-p7	Steatosis, Oxidative stress, Carcinoma (rare)	Albumin	(+) Immunohistochemistry	C57BL/6	Lerat *et al.*[19]
Full length	Steatosis, Oxidative stress, Carcinoma	Albumin	(−) Immunohistochemistry	C57BL/6	Lerat *et al.*[19]
Core E1-E2-p7-NS2	Substantial hepatic pathology, Presence of Anti-Core antibodies, HCV-specific CD8+ cytotoxic T lymphocyte response, Alanine aminotransferase increase, Inhibition of Fas-mediated apoptosis	LoxP/chicken beta-actin with CMV-IE enhancer	(+) Western Blot (+) Immunohistochemistry	Balb/C	Wakita *et al.*[21,22] Machida *et al.*[23]
Core	CD8+ cytotoxic T lymphocyte suppression, Reduced production of IFNγ and IL-2	Vaccinia Virus	(+) Western Blot	Balb/C	Large *et al.*[24]

from mitochondria.[23] Inhibition of the Fas signalling pathway by HCV, allowing interference with cytotoxic T-cell-mediated elimination of infected cells, could explain the capacity of the virus to escape the host immune system. Interestingly, a suppression of CTL immune response was also observed in mice infected with a chimeric vaccinia/HCV core virus, which represents an alternative model to mimic the acute phase of HCV infection.[24] A limitation of the inducible transgenic approach described above is the boost of the immune response induced by the adenovirus used to express the Cre recombinase, which could influence the magnitude of the responses to HCV protein expression. The use of more neutral inducible systems such as, for example, tetracycline-inducible expression, should allow a more rigorous analysis of the phenotype of acute infection.

In summary, no unequivocal conclusion about a direct role of viral proteins in liver pathology, caused by HCV, can be drawn from these reports. The variability of the phenotypes observed can be partially explained by the use both of mice with different genetic backgrounds and of distinct promoters driving different temporal-spatial levels of transgene expression. Moreover, a direct comparison between different transgenic mice is made very difficult by the heterogeneity of the parameters analysed by the different groups. More detailed studies on HCV transgenic models, describing systematically all parameters that may recapitulate human traits of HCV-associated pathogenesis are therefore desirable, since, particularly in this case, negative and positive results can be equally informative.

Nevertheless, even considering the heterogeneity of the morphological observations reported, biochemical studies in transgenic mice, further corroborated by *in vitro* analyses, strongly suggest a direct role of the core protein in HCV-related pathogenesis. In fact, core has been proposed to cause a direct inhibition of the microsomal triglyceride transfer protein activity resulting in modification of very low density lipoprotein assembly and secretion; this provides a putative mechanism for the onset of steatosis.[25] Moreover, core has been shown to alter mitochondrial function increasing production of reactive oxygen species.[11,20] Accordingly, these effects might directly contribute to the onset of steatosis, fibrosis, and, via oxidative DNA damage, predispose to the development of hepatocellular carcinoma. These studies may offer a new theoretical basis for the development of specific therapies for HCV pathogenesis.

Recently, a significant breakthrough in the development of small animal models for HCV infection has been obtained by Mercer *et al*.[26] which developed a very efficient model of human hepatocyte transplantation in mice. They utilized the Alb-uPA transgenic mice carrying the urokinase-type plasminogen activator gene under the control of the albumin promoter. The transgene expression leads to hepatocyte death providing an environment suitable for the engraftment of non-transgenic cells. Backcrossing Alb-uPA mice into the SCID background, then generated mice prone to xenotransplantation. The authors successfully infected these transplanted mice with the serum of HCV-positive patients. HCV infection was persistent, viral RNA was detectable up to 35 weeks post infection and, relevantly, the HCV infection can be serially passaged through generations by serum injection.[26] This new model potentially represents a powerful tool to perform several new studies, first of all allowing testing of the effectiveness of putative antiviral drugs and gene therapeutic strategies. Furthermore, different aspects of the HCV life cycle could be exploited analysing *in vivo* the effects of introducing specific mutations in the viral genome. From a pathological point of view, knowing that HCV takes a long period of time to induce cytopathic effects, the capability of these chimeric mice to sustain a long-term HCV infection remains to be evaluated. Moreover, the lack of an immune system in this model represents a limitation in mimicking the real environment of a HCV infection. Theoretically this problem could be solved using human bone marrow cells as donor in immune reconstitution experiments.

In conclusion, in spite of the many animal models so far generated, the final goal of these studies, i.e. the comprehension of the role of each viral product in HCV-related pathogenesis, is far from being fulfilled. To conclusively unveil HCV pathogenesis a genetically engineerable experimental model supporting the entire life cycle of the virus will have to be established. The number and nature of the limiting factors on this route are still a guess. The recent availability of cell culture systems for HCV studies could allow, in the near future, identification of species-specific cellular factors required for HCV infection and replication.[27] In principle, the expression of these factors in transgenic mice, could allow breakage of the host-specificity of the virus and ultimately provide a suitable experimental system to mimic the various aspects of HCV infection.

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