A TRANSGENIC MOUSE MODEL OF STEATOSIS AND HEPATOCELLULAR CARCINOMA ASSOCIATED WITH CHRONIC HEPATITIS C VIRUS INFECTION IN HUMANS

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INTRODUCTION

Chronic hepatitis C is a necroinflammatory disease of the liver that is caused by infection with hepatitis C virus (HCV) (1). Uniquely adapted to establish persistent infections within the human liver, the major pathologic hallmark of this flavivirus infection is the insidious progression of hepatic fibrosis and loss of functioning hepatocyte mass (2–4). Typically, this occurs against a background of chronic hepatic inflammation and associated macro- and microvesicular steatosis. A significant proportion of infected persons ultimately develop cirrhosis, hepatic failure, or hepatocellular carcinoma (HCC) (5–7). These lethal manifestations of hepatitis C are currently thought to result in approximately 8,000–10,000 deaths annually among the 3.9 million Americans infected with this virus (8). This makes hepatitis C the single most common cause of clinically significant liver disease in the United States today. Despite this, little is known about the molecular mechanisms underlying the chronic hepatic changes that are associated with this viral infection. However, it is generally believed that most if not all of the pathologic consequences of hepatitis C arise as a result of the cellular immune response to the infection, and that the infection itself is likely to be noncytopathic.

Existing therapies for chronic hepatitis C include recombinant human interferon, either alone or in combination with ribavirin (9,10). These antiviral therapies are only partially effective, leading to the

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resolution of infection in no more than about 40% of treated persons. They are also accompanied by a high incidence of adverse side effects. Thus, there is an urgent need to develop safer and more effective therapeutics for treatment of hepatitis C. However, drug discovery has been stymied by two overwhelming technical deficiencies. These include, first, the absence of a cell culture system that is permissive for efficient HCV replication and, second, the lack of small animal models that either support replication of HCV or mimic aspects of the pathogenesis of chronic hepatitis C in humans. The chimpanzee (*Pan troglodytes*) is the only animal species that has been shown clearly to be permissive for HCV infection, and infected chimpanzees typically develop little evidence of liver disease as a result of infection with the virus (11,12).

Here, we present a preliminary report of studies aimed at addressing the second of these technical deficiencies, the lack of readily available animal models of hepatitis C. These studies involve the development and characterization of transgenic mice that express HCV proteins and/or viral RNAs in a liver-specific fashion. Some of these transgenic lineages appear to develop hepatic pathology similar to that observed in human disease, and thus may be useful in unraveling the complex pathobiology of this disease. These animals may also offer unique opportunities for preclinical studies of novel therapies for chronic hepatitis C.

MATERIALS AND METHODS

Construction of HCV transgenes and development of transgenic mice. We established multiple independent lineages of transgenic mice that harbor as a transgene cDNA corresponding to the full-length open reading frame (ORF) of a Japanese genotype 1b virus, HCV-N (13). The sequence of this transgene was derived from the plasmid pMN2-1G (14). The expressed HCV RNA segment is approximately 9 kb in length, and encodes a polyprotein of just over 3000 amino acids that is proteolytically processed into 10 distinct viral proteins by both host cell and viral proteinases acting both *in cis* and *in trans* (15). To provide liver-specific expression, the transgene was placed under the control of the murine albumin promoter-enhancer (16), and immediately upstream of the poly(A) intron of SV40 virus. The vector used for construction of the transgene was pGEMAlbSVPA. Transgenic mice were created using standard techniques within a C3H/C57BL6 genetic background. Founder animals (F_0) were identified by Southern blotting of DNA isolated from tail biopsies using an HCV-specific cDNA probe.

Breeding capacity. F_0 founder animals were crossed with normal C57BL/6 mice to produce F_1 offspring in order to evaluate breeding potential as well as the level of HCV gene expression. The frequency of transgene transmission to offspring was determined by Southern blotting or PCR amplification of DNA extracted from tail biopsy specimens.

RNA transcription. RNA transcription was evaluated in F_1 offspring by northern analysis and by a reverse-transcription polymerase chain reaction (RT-PCR) followed by Southern hybridization of the amplified products. For RT-PCR, stringent controls ensured that the PCR amplification of HCV sequence was from RNA transcripts and not the cDNA transgene itself. Total RNA extracted from mouse liver was purified by two cycles of RQ1 DNase digestion, each followed by LiCl precipitation of RNA. Parallel, no-reverse transcriptase control reactions were included in each assay to document that the template for amplification products was RNA and not DNA.

HCV protein expression. A variety of standard techniques were used for the detection of HCV proteins within the livers of transgenic mice, including indirect immunofluorescence with rabbit polyclonal antibodies to the viral core protein and the hypervariable region of the E2 protein of HCV. We also attempted immunoblot detection of the core protein using murine monoclonal antibodies to these proteins, following immunoprecipitation from mouse liver extracts with rabbit polyclonal antibodies specific for core.

RESULTS

HCV transgenic mice with liver-specific expression of the HCV ORF. A total of 15 F_0 founder animals were shown by Southern analysis to harbor as a transgene the full-length ORF of genotype 1b HCV (FL-N transgene) (data not shown). However, the F_1 offspring from these lineages were uniformly negative when tested by northern analysis for the presence of HCV RNA transcripts within the liver. It is likely that the absence of founder animals with efficient transcription of the transgene is due to the length of the expected transcript (about 9 kb), as this may be associated with difficulties in nuclear export of the RNA. Nonetheless, livers from 4 of these 15 FL-N transgenic lineages contained HCV-specific RNA transcripts that were detectable by RT-PCR (Fig. 1). These transcripts were only detected when reverse transcription was carried out on the extracted RNA samples prior to PCR, demonstrating that the product obtained in these reactions was derived from RNA. Moreover, RNA transcripts in

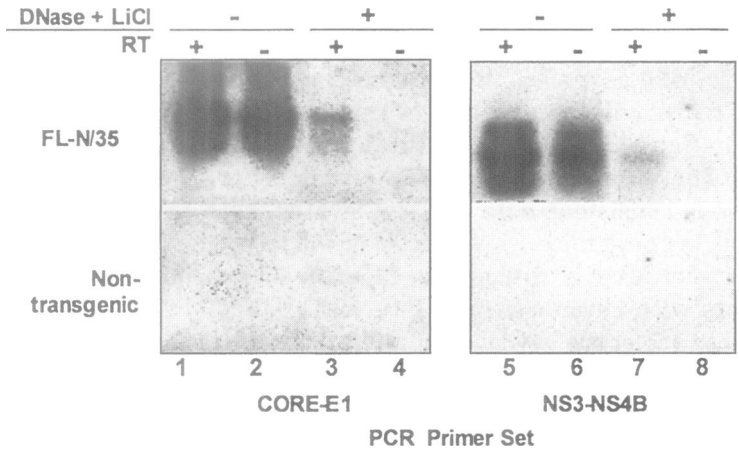


FIG. 1. Southern hybridization of PCR reaction products obtained with total RNA from liver of an FL-N/35 mouse (top panels) or a nontransgenic mouse (bottom panels). Lanes 1–4 contain products amplified with PCR primers spanning the core-E1 region, and lanes 5–8 with primers spanning NS3-NS4B. RNA in lanes 3, 4, 7, 8 was subjected to two cycles of Dnase followed by LiCl precipitation, while RNA in lanes 1, 2, 5, 6 was not. Products arise from the contaminating cDNA transgene in the latter, but not the former. Lanes 1, 3, 5, and 7 contain products amplified following reverse transcription, while lanes 2, 4, 6 and 8 are RT(−) reaction controls.

the FL-N/35 lineage were detected with multiple PCR primer sets amplifying overlapping segments that span the entire HCV polyprotein from the core protein to NS5B (data not shown). With each primer set, there were only single PCR amplimers of the expected size. These results suggest that the entire ORF is expressed without nuclear splicing of the RNA. This is important, since splicing has been noted with other transgenes derived from HCV genomic sequences (C. Pasquinelli, personal communication).

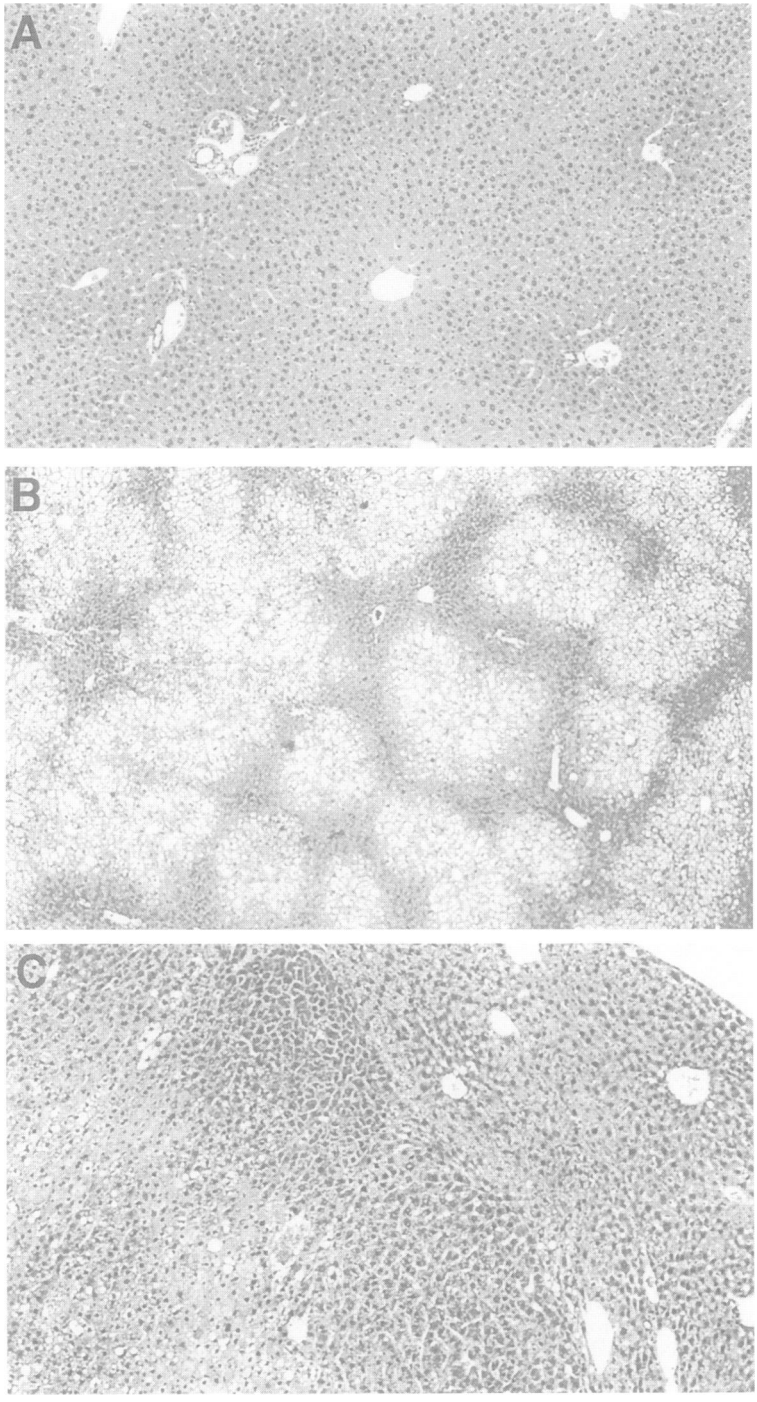
Of the 4 transgenic lineages with detectable HCV transcripts, only one (FL-N/35) has been bred successfully in large numbers (see below). The frequency of transgene transmission in this lineage approximates 50%, consistent with the heterozygous nature of the transgene and the results of Southern blotting which suggest a single transgene integration site.

HCV protein expression. Evidence of HCV core and E2 protein expression was sought in the livers of mice from the FL-N/35 lineage. Despite the fact that these mice were reproducibly positive for viral RNA in the RT-PCR assays described above, we were unable to detect these proteins by indirect immunofluorescence or immunoprecipitation followed by immunoblotting. Positive controls for these experi-

ments included stably transformed Chinese hamster ovary (CHO) cells that constitutively express these HCV proteins (M. Honda et al., in preparation), as well as liver taken from other, more recently established transgenic mouse lineages that express the segment of the viral genome that encodes the structural proteins of the virus (unpublished data). The absence of detectable protein expression in the FL-N/35 lineage is consistent with the low level of RNA transcription that is evident in these mice. However, it is worth noting that HCV proteins are extraordinarily difficult to identify in liver biopsies from most patients with chronic hepatitis C, and that RT-PCR is routinely required to detect the low levels of RNA transcripts that patients harbor.

Disease phenotypes expressed by HCV transgenic mice. The FL-N/35 lineage is the only transgenic line for which we have extensive data concerning the frequency of liver injury associated with transgene expression. Importantly, there was no cellular inflammatory infiltrate present evident within the livers of either these transgenic animals or their nontransgenic littermates. The absence of inflammation is consistent with the prenatal expression of transgenic proteins that are placed under control of the mouse albumin promoter, and the likelihood that these mice are thus immunologically tolerant to the HCV proteins they express. However, animals from the FL-N/35 lineage do show age- and gender-related microvesicular steatosis, as well as an increased frequency of HCC. Although we do not have reliable data on the frequency of these pathologies in other transgenic lineages, we have observed both hepatocellular carcinoma and steatosis among the small numbers of animals we have necropsied thus far from other FL-N transgenic lineages. This strongly suggests that the findings in the FL-N/35 lineage are not due to disruption of a critical mouse gene by fortuitous integration of the transgene.

Microvesicular Steatosis. Histopathological analysis demonstrated a profound microvesicular steatosis in livers from older mice in the FL-N/35 lineage. Similar pathology was present in animals from an independently-derived transgenic mouse line expressing the same transgene, FL-N/986. Milder steatosis was seen in normal mice of the same age. Fig. 2 demonstrates the findings. Fig. 2A shows liver taken from a normal, 10-month old nontransgenic male, while Fig. 2B demonstrates microvesicular steatosis in an FL-N/35 transgenic male of similar age. In the transgenic animal, steatosis is present exclusively in a pericentral distribution involving zones 2 and 3. There is no involvement of the periportal zone 1. The steatosis is predominantly microvesicular in nature, but some larger lipid droplets are also evident at higher magnification.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We quantified the degree of steatosis by staining frozen sections with oil red O and using a digital image analysis program to measure the percent area occupied by the fat droplets in 3 separate fields from each microscopic tissue section. Transgenic animals were compared with nontransgenic animals in the colony. These experiments demonstrated that transgenic mice aged 9–15 months had greater steatosis than age-matched, non-transgenic controls. Steatosis was more severe in older animals, especially males. In contrast, steatosis was present, but both less common and milder in transgenic females. A lesser degree of steatosis was also present in older nontransgenic males but, importantly, no steatosis was ever observed in nontransgenic females. The steatosis in the FL/N transgenic animals mimics the steatosis observed in HCV-infected humans (17–19), and suggests that this manifestation of hepatitis C may be caused directly by the expression of one or more HCV proteins.

Hepatocellular carcinoma (HCC). Well-differentiated HCC were found in approximately one third of F_1 FL-N/35 animals undergoing necropsy at greater than 12 months of age. Some tumors were large, greater than 1 cm in diameter, and demonstrated distinct compression of adjacent nontumor tissue (Fig. 2C). We have not necropsied sufficient numbers of animals to know the frequency of HCC in the other FL-N transgenic lineages. However, we have documented the occurrence of well-differentiated HCC in one older mouse derived from another, independent FL-N lineage (FL-N/984). HCC occurred exclusively among male transgenic animals, and was never present in transgenic animals less than 13 months of age, or in nontransgenic littermates of any age. As with steatosis, these observations suggest a direct or indirect effect of HCV protein expression on the development of liver cancer independent of the liver inflammation that typifies chronic hepatitis C.

DISCUSSION

Our work has focused on the development of transgenic mice that constitutively express HCV proteins in a liver-specific fashion. Other

Fig. 2. Histopathology of liver sections from transgenic and normal, nontransgenic animals in the mouse colony. (A) A 10-month old nontransgenic male mouse showing normal liver histology. (B) A similarly aged FL-N/35 male animal showing extensive microvesicular steatosis involving exclusively Rapoport zones 2 and 3. There is no cellular infiltrate or other evidence of inflammation or cellular necrosis. (C) A well differentiated hepatocellular carcinoma with compression of normal adjacent liver tissue in a 13 month old male from the FL-N/35 lineage.

investigators have also developed HCV transgenic animals (20–23), but our efforts are unique in that they have begun to reveal the histological characteristics of animals that constitutively express low levels of HCV transcripts encoding the complete viral polyprotein. We have found increased micro- and macrovesicular hepatic steatosis and an increased incidence of HCC in male transgenic mice (FL-N/35 lineage). These are common histologic features of hepatitis C in humans (19). The absence of inflammation in our transgenic animals is consistent with the likelihood that these animals are immunologically tolerant to the HCV proteins they express, as the transgene is under the transcriptional control of the albumin promoter and thus likely to be expressed during fetal development. The lack of hepatic inflammation clearly distinguishes these animals from most humans with chronic hepatitis C. Thus, an important conclusion from these studies is that both steatosis and cancer may develop as a direct or indirect result of the expression of one or more HCV proteins within the liver. These manifestations of hepatitis C may not arise strictly as a result of immune-mediated inflammation, as often assumed, especially for hepatocellular carcinoma.

As in humans, we found liver cancer to be much more frequent in transgenic males than in females. Koike and colleagues (21,24) have also described steatosis and HCC in older male HCV transgenic mice. However, their findings differ from ours in several important respects. First, their transgenic animals expressed only the core protein of HCV, not the entire polyprotein. Furthermore, they appear to express this protein at a very high abundance (readily detectable in immunoblots) compared with the level of expression apparent in our FL-N/35 lineage. In addition, HCC developed at a somewhat older age in these mice than those in our FL-N/35 lineage. Interestingly, two other groups of investigators, Kawamura *et al.* (22) and Pasquinelli *et al.* (23) have found no pathology in transgenic mice expressing the core or E2 proteins of HCV. The reasons underlying these differences are unknown, but they cannot be explained by insufficient levels of expression or the specific genotype of HCV (1a *vs.* 1b) in these mice. The most interesting lead is that our transgenic mice and those developed by Koike's group (21,24) are in a C57BL/6 genetic background, while this was not the case for those studied by Kawamura *et al.* (22) or Pasquinelli *et al.* (23). Thus, genetic background might be an important factor in determining disease expression in HCV transgenic mice. If so, further study of these differences might ultimately provide some clues as to why some infected humans develop severe liver disease, while others do not.

While we do not know which HCV proteins (structural vs. nonstructural) are responsible for steatosis and HCC in our FL-N transgenic lineages, it is likely that the core protein is involved since steatosis and cancer were both observed by Moriya *et al.* in transgenic mice expressing only this protein (21,24). Two hypotheses can be envisioned as to how core might cause such pathology. Barba *et al.* (25) suggested that the HCV core protein physically associates with lipid-laden vesicles and that this alters normal lipid metabolism and/or transport leading to steatosis. An alternative hypothesis is suggested by the observation that core protein interacts with the cytoplasmic extension of the tumor necrosis factor receptor 1 (TNFR-1) and related members of this receptor family (26,27). In vitro studies investigating the potential biologic consequences of this interaction have led to conflicting results (28–31). However, in the transgenic mouse as well as the liver of infected humans, this interaction could constitutively activate proapoptotic signaling, leading to upstream caspase activation, a mitochondrial permeability transition, uncoupling of oxidative phosphorylation, the accumulation of free radicals, oxidative stress and lipid peroxidation. While speculative, the centrilobular (zone 3) microvesicular steatosis in our mice is very consistent with mitochondrial injury and makes this an attractive hypothesis. Further studies will be needed to document whether or not this is the case.

The specific mechanism of carcinogenesis in these transgenic animals is also unknown. It could relate to a transforming action of the core protein, as suggested by some *in vitro* studies (32). Dysregulation of cell growth could also be due to the interaction of core with TNFR-1, as antiapoptotic regulatory mechanisms could be activated by constitutive signaling through the receptor. Roles in cellular carcinogenesis have also been suggested for both the HCV NS3 and NS5A proteins (33,34), however, and all of these proteins are likely to be expressed at low levels in our transgenic mice. Alternatively, chronic oxidative stress may set the stage for carcinogenesis in these mice by promoting conditions for chromosomal DNA damage.

CONCLUSIONS

The extent to which the mechanism of carcinogenesis in these animals relates to the development of liver cancer in infected humans remains to be established. It is very likely that chronic inflammation plays an important role in the development of HCC in chronic hepatitis C, as it does in chronic hepatitis B. However, the results described here strongly suggest that the intrahepatic expression of one or more HCV

proteins significantly enhances the risk of cancer, even in the absence of an immunologically mediated inflammatory response to the infection.

REFERENCES

1. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244:359–362.
2. Alter MJ, Margolis HS, Krawczynski K, et al. The natural history of community-acquired hepatitis C in the United States. N. Engl. J. Med. 1992; 327:1899–1905.
3. Seeff LB, Buskell-Bales Z, Wright EC, et al. Long-term mortality after transfusion-associated non-A, non-B hepatitis. N. Engl. J. Med. 1992; 327:1906–1911.
4. Seeff LB. Natural history of hepatitis C. Hepatology 1997; 26:21S–28S.
5. Di Bisceglie AM, Simpson LH, Lotze MT, Hoofnagle JH. Development of hepatocellular carcinoma among patients with chronic liver disease due to hepatitis C viral infection. J. Clin. Gastroenterol. 1994; 19:222–226.
6. Kiyosawa K, Furuta S. Hepatitis C virus and hepatocellular carcinoma. In: Reesink HW, editor. Hepatitis C Virus. Basel: Karger, 1994:98–120.
7. Kiyosawa K, Sodeyama T, Tanaka E, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 1990; 12:671–675.
8. Alter MJ, Mast EE, Moyer LA, Margolis HS. Hepatitis C. Infect. Dis. Clin. North Am. 1998; 12:13–26.
9. Di Bisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alfa therapy for chronic hepatitis C: A randomized, double-blind, placebo-controlled trial. N. Engl. J. Med. 1989; 321:1506–1510.
10. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N. Engl. J. Med. 1998; 339:1485–1492.
11. Alter HJ, Holland PV, Purcell RH, Popper H. Transmissible agent in non-A, non-B hepatitis. Lancet 1978; i:459–463.
12. Tabor E, Gerety RJ, Drucker JA, et al. Transmission of non-A, non-B hepatitis from man to chimpanzee. Lancet 1978; i:463–466.
13. Hayashi N, Higashi H, Kaminaka K, et al. Molecular cloning and heterogeneity of the human hepatitis C virus (HCV) genome. J. Hepatol. 1993; 17:S94–107.
14. Honda M, Ping L-H, Rijnbrand RCA, et al. Structural requirements for initiation of translation by internal ribosomal entry within genome-length hepatitis C virus RNA. Virology 1996; 222:31–42.
15. Major ME, Feinstone SM. The molecular virology of hepatitis C. Hepatology 1997; 25:1527–1538.
16. McPherson CE, Shim E-Y, Friedman DS, Zaret KS. An active, tissue-specific enhancer and bound transcription factors existing in a precisely positioned nucleosomal array. Cell 1993; 75:387–398.
17. Hourigan LF, Macdonald GA, Purdie D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999; 29:1215–1219.
18. Czaja AJ, Carpenter HA, Santrach PJ, Moore SB. Host- and disease-specific factors affecting steatosis in chronic hepatitis C. J. Hepatol. 1998; 29:198–206.
19. Goodman ZD, Ishak KG. Histopathology of hepatitis C virus infection. Semin. Liver

Dis. 1995; 15:70–81.
20. Koike K, Moriya K, Ishibashi K, et al. Expression of hepatitis C virus envelope proteins in transgenic mice. J. Gen. Virol. 1995; 76:3031–3038.
21. Moriya K, Yotsuyanagi H, Shintani Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J. Gen. Virol. 1997; 78:1527–1531.
22. Kawamura T, Furusaka A, Koziel MJ, et al. Transgenic expression of hepatitis C virus structural proteins in the mouse. Hepatology 1997; 25:1014–1021.
23. Pasquinelli C, Shoenberger JM, Chung J, et al. Hepatitis C virus core and E2 protein expression in transgenic mice. Hepatology 1997; 25:719–727.
24. Moriya K, Fujie H, Shintani Y, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat. Med. 1998; 4:1065–1067.
25. Barba G, Harper F, Harada T, et al. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proceedings of the National Academy of Sciences of the United States of America 1997; 94:1200–1205.
26. Matsumoto M, Hsieh TY, Zhu NL, et al. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotoxin-β receptor. J. Virol. 1997; 71:1301–1309.
27. Chen CM, You LR, Hwang LH, Lee YH. Direct interaction of hepatitis C virus core protein with the cellular lymphotoxin-beta receptor modulates the signal pathway of the lymphotoxin-beta receptor. J. Virol. 1997; 71:9417–9426.
28. Ray RB, Meyer K, Ray R. Suppression of apoptotic cell death by hepatitis C virus core protein. Virology 1996; 226:176–182.
29. Ray RB, Meyer K, Steele R, Shrivastava A, Aggarwal BB, Ray R. Inhibition of tumor necrosis factor (TNF-alpha)-mediated apoptosis by hepatitis C virus core protein. J. Biol. Chem. 1998; 273:2256–2259.
30. Zhu N, Khoshnan A, Schneider R, et al. Hepatitis C virus core protein binds to the cytoplasmic domain of tumor necrosis factor (TNF) receptor 1 and enhances TNF-induced apoptosis. J. Virol. 1998; 72:3691–3697.
31. Ruggieri A, Harada T, Matsuura Y, Miyamura T. Sensitization to Fas-mediated apoptosis by hepatitis C virus core protein. Virology 1997; 229:68–76.
32. Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis C virus core protein cooperates with *ras* and transforms primary rat embryo fibroblasts to tumorigenic phenotype. J. Virol. 1996; 70:4438–4443.
33. Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. J. Virol. 1995; 69:3893–3896.
34. Gale MJ, Kwieciszewski B, Dossett M, Nakao H, Katze MG. Antiapoptotic and oncogenic potentials of hepatitis C virus are linked to interferon resistance by viral repression of the PKR protein kinase. J. Virol. 1999; 73:6506–6516.

DISCUSSION

WILLIAMS, Albequerque: This is a really interesting model that you have developed and I wonder if these mice have any auto antibodies or whether there is any cryoglobulin produced? Do transgenic mice have an abnormal immune system?

LEMON: We are just beginning to look at that. An interesting question would be whether these mice have difficulty eliminating an adenoviral infection from their liver. We don't understand why hepatitis C virus persists. However, I would doubt that they have cryoglobulins because that is usually associated with an anti-envelope antibody response. We haven't measured that in these animals, but we would not expect to find that.

BLOOMER, Birmingham: In the human with HCV infection there seems to be a poor correlation, if any, between the level of viral replication and hepatocellular damage assessed by light microscopy. Yet when there is active viral replication, it is presumed you are making more of these proteins that you are studying in your animal. I wonder how you would explain that in human disease?

LEMON: Well, there are several ways to look at that. One is that when we talk about viral levels we are talking about viremia. We are measuring virus in the blood and not virus in the liver, which is where the virus load really is. The amount of virus in the blood is probably determined in part by how much anti-envelope antibody is present and how rapidly it is being cleared. We really don't understand those kinetics very well. One would also have to look at the amount of virus that one finds in the blood as well as the liver as a result of a balance between replication and the immune response to it. If the immune response is playing a primary or important role, certainly a contributing role to pathogenesis, then you would not expect a direct correlation between virus in the blood and damage in the liver.

STEVENSON, Stanford: This is becoming a perinatal problem as well. Not much is known about that yet in terms of transmission, but one of the questions that might relate to the work that you are doing and that I have is this: as experience is gained, unfortunately, are people beginning to look at the time of onset of tumor in the perinatally exposed and infected individuals compared to the individuals that get it later? Are there clues there as to what might be going on in terms of the mechanism, independent of the aging of the individual?

LEMON: A short answer is, no, no one is studying that. I think hepatitis C in the pediatric population is something that really needs to be studied much more than is the case. It is apparent that in Japan cancer is a much more common form of death than cirrhosis. In this country it seems to be just the opposite. We don't understand the basis of that at all, but it is probably not strictly genetic. There are more questions than answers for sure.

MADDREY, Dallas: Stan, you have presented very interesting findings in that you did not observe much inflammation and you have shown a relatively quick onset of tumor. In man, almost every case of hepatitis C-related cancer occurs in a patient who has cirrhosis. A further observation that has come from Japan is that even in patients with cirrhosis, a modest amount of Interferon for a long period of time is anti-carcinogenic. It seems to me that your model will not only lend itself to looking at the dissociation between inflammation and progression to cirrhosis and carcinogenesis, but also offer a possible other model for looking at some of the effects of interferon, such as the possible anti-angiogenesis effect in preventing tumor.

LEMON: I would agree with all those statements, Willis. Interferon clearly does have a protective effect against the development of cancer in these patients and the Japanese were first to note that. We don't understand the mechanism, however. Interferon probably is acting via a primary anti-viral effect which would reduce protein expression. It is also clearly moderating inflammation. These features of the interferon response may or may not be related.

As to the statement that these animals are developing tumors rapidly, I don't think that is quite the case. We are looking at tumors at 13 to 18 months of age in these animals, which is at least half the life span of the mouse.