New Animal Models of Hepatitis B and C

Mark A. Feitelson and Jonathan D. Larkin

Abstract

The narrow host range of infection and lack of suitable tissue culture systems for the propagation of hepatitis B and C viruses are limitations that have prevented a more thorough understanding of persistent infection and the pathogenesis of chronic liver disease. With hepatitis B virus (HBV), this lack of knowledge has been partially overcome by the discovery and characterization of HBV-like viruses in wild animals. With hepatitis C virus (HCV), related flaviviruses have been used as surrogate systems for such studies. Other laboratories have developed transgenic mice that express virus gene products and/or support virus replication. Some HBV transgenic mouse models develop fulminant hepatitis, acute hepatitis, or chronic liver disease after adoptive transfer, and others spontaneously develop hepatocellular carcinoma (HCC), as in human infections. Among HCV transgenic mice, most develop no disease, but acute hepatitis has been observed in one model, and HCC in another. Although mice are not susceptible to HBV and HCV, their ability to replicate these viruses and to develop liver diseases characteristic of human infections provides new opportunities to study pathogenesis and develop novel therapeutics.

Key Words: animal models; hepatitis B virus; hepatitis C virus; pathogenesis; transgenic mice

Introduction

More than 350 million people worldwide are chronic carriers of hepatitis B virus (HBV), and approximately 170 million are chronically infected with hepatitis C virus (HCV) (Delwaide and Gerard 2000; Wild and Hall 2000). These people are at high risk for the development of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). HCC is one of the most frequent tumor types worldwide, with a yearly incidence of up to one million (Esquivel et al. 1999). Both cirrhosis and HCC are major causes of mortality within 20 to 40 yr after infection (Alter 1996). Although screening tests have now nearly eliminated these viruses from the blood supply (Gerlich and Caspari 1999), significant transmission still occurs sexually (for HBV) (Mast et al. 1998) and through intravenous drug abuse (for HBV and HCV) (Maddrey 2000; Smyth et al. 1995). Thus, both viruses remain very important public health threats worldwide.

Treatment for chronic infections is limited, with interferon resulting in a sustained response in only about 20% of HBV or HCV patients (Lindsay 1997; Torresi and Locarnini 2000). New therapies have recently been adopted with the introduction of lamivudine for HBV (Dusheiko 1999) and ribavirin for HCV (Reichard et al. 1997). Lamivudine is highly effective against HBV in most patients, while the combination of interferon plus ribavirin typically shows a sustained virological and histopathological response in up to 50% of HCV-infected patients after the end of treatment. Prolonged treatment with interferon has considerable side effects (Hoofnagle and Lau 1996), and lamivudine-resistant HBV has been detected in up to 20% of patients after 1 yr of treatment (Honkoop et al. 1997; Tipples et al. 1996). Given the half billion or more people chronically infected with these viruses, there is a strong mandate to develop relevant animal models to test new drug candidates to combat both of them and their associated liver diseases.

Natural Hosts for HBV and HCV

Part of the difficulty in developing new therapeutic approaches stems from a fundamental lack in understanding the pathogenesis of chronic liver disease (CLD) associated with these viral infections (Figures 1 and 2). Chimpanzees have been invaluable for studying the transmission and natural history of infection, have provided important information about pathogenesis, and have been used to conduct vaccine studies (Prince and Brotman 1998; Tabor et al. 1983). In the case of HCV, chimpanzees were central to the discovery of the virus (Alter et al. 1978; Bradley et al. 1979). However, their availability is limited due to their expense and endangered status. In addition, the liver disease in experimentally infected chimpanzees is characteristically mild, which makes it impossible...
Acute HBV infection

- 10% Weak Th1 & CTL response
- 70-90% Chronic infection (HBsAg + for > 6 mo.)
- 10-30% Asymptomatic chronic carrier (no liver disease)
- 25% Strong Th1 & CTL response
- 65% Acute hepatitis

Fulminant hepatitis (< 1%)
- Death or recovery
- Resolution & recovery
- Regression
- Cirrhosis
- Hepatocellular carcinoma

Figure 1 Pathogenesis and outcomes of hepatitis B virus (HBV) infection. Upon acute exposure to HBV, the majority of adults (~65%) develop a subclinical infection that is indicated only by the appearance of one or more viral antibodies. Another roughly 25% of infected adults develop a strong T helper type 1 (TH1) cytokine and cytotoxic T lymphocyte (CTL) response, which results in a bout of acute hepatitis followed by resolution and recovery. Rarely, an acute infection develops rapidly into life-threatening fulminant hepatitis. Approximately 10% of acutely infected adults develop a chronic infection, which is characterized by the persistence of HBsAg and (in some patients) virus in blood. Although most chronic carriers remain asymptomatic for years or decades, they are at great risk for the development of chronic hepatitis, cirrhosis, and hepatocellular. Among those who develop liver disease, progression is variable, with some patients developing end-stage liver disease or hepatocellular within a few years, while others undergo regression of histological lesions in the liver at any stage of chronic liver disease.

Hepadnaviruses

Hepadnaviruses (Robinson et al. 1982) include HBV and a growing number of agents that naturally infect selected hosts in the wild. The three best studied are the ground squirrel hepatitis virus, which infects ground squirrels (Marion et al. 1980), the woodchuck hepatitis virus, which infects woodchucks (Summers et al. 1978), and the duck hepatitis B virus (DHBV1), which infects Pekin ducks (Mason et al. 1980). Mammalian hepadnaviruses have a genetic organization similar to that of HBV (Figure 3), while DHBV lacks the X gene. Infected ducks have been very useful in elucidating the replication scheme of hepadnaviruses (Summers and Mason 1982), and primary duck hepatocytes are readily infected with DHBV (Pugh and Summers 1989). This makes them particularly useful for the evaluation of drugs that inhibit virus replication in a fully permissive in vitro system (Colledge et al. 1997; Schulz and Chisari 1999). Infected ducks and woodchucks have also been exploited in the area of preclinical antiviral drug development (Hurwitz et al. 1998; Mason et al. 1994), having become standard in vivo to study the pathogenesis of cirrhosis or HCC. For these reasons, it would not be practical to use chimpanzees for the preclinical evaluation of new therapeutic approaches on a regular basis, or for the evaluation of combination therapies.

Recently, the tree shrew (Tupaia) has been shown to be susceptible to HBV and HCV infections (Xie et al. 1998; Yan et al. 1996a,b). Transmission has been readily achieved using HBV-infected animals and has been prevented by prior immunization of susceptible animals with the HBV vaccine. Chronically infected animals developed a low incidence of HCC, as did uninfected animals treated with aflatoxin B1, although the frequency of HCC was more than 50% among infected animals given aflatoxin. Among HCV-infected animals, viremia was transient or intermittent in about one third of the infected animals, and for a more extended time in half the animals that were immunosuppressed by irradiation before infection. Although this small animal model holds promise as being susceptible to infection by these viruses, the model is relatively uncharacterized, and only future work will determine whether it will provide any significant advantages over the chimpanzee.

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Acute HCV infection

Weak CTL response
strong Th2 response I 85%

Strong CTL response
strong Th1 response I 15%

Chronic HCV infection

Mild and moderate immune responses

Strong immune responses

Chronic hepatitis

Cirrhosis

End-stage liver disease

HCC

Figure 2 Pathogenesis and outcomes of hepatitis C virus (HCV) infection. Acute HCV infection has two major outcomes. Patients who develop strong, rapid, multispecific T cell responses often experience either a clinical or subclinical bout of acute hepatitis, followed by resolution, or no liver disease at all. However, the majority of acutely infected patients develop persistent viremia, frequently characterized by the appearance of chronic hepatitis. The progression or development of cirrhosis during chronic infection depends on the characteristics of the immune responses that develop, with more vigorous sustained immune responses associated with increased liver damage and disease progression. Alternatively, the triggering of weak cell-mediated immune responses after acute infection, or during the course of chronic infection, would favor the development of virus escape mutants that would persist. CTL, cytotoxic T lymphocyte; HCC, hepatocellular carcinoma. Modified from Hoofnagle JH. 1997. Hepatitis C: The clinical spectrum of disease. Hepatology 26(Suppl 1): 15-20.

models for preclinical evaluation of nucleoside analogs with potential activity against HBV. Infected woodchucks have also been used in basic studies focused on the natural history of infection (Korba et al. 1989, 1990), which is similar to that in chronic human infections (Figure 1). Chronically infected woodchucks have also been useful in studying the pathogenesis and molecular mechanisms of CLD and HCC (Fourel et al. 1994; Hsu et al. 1988; Popper et al. 1987; Snyder et al. 1982). Even with these strengths, the lack of inbred ducks and/or woodchucks, as well as the lack of duck- and woodchuck-specific reagents to analyze the immune cells important to the pathogenesis of acute and chronic infections, remain formidable obstacles in understanding the molecular and cellular basis of disease. In addition, neither infected ducks nor woodchucks develop cirrhosis, nor do infected ducks develop HCC. In addition, given that the pathogenesis of infection with these viruses is immune mediated, immunological reagents that might have therapeutic efficacy against the woodchuck or duck viruses would probably not be useful against HBV (e.g., a monoclonal antibody), and that for human trials, HBV-specific reagents would have to be created and tested. Hence, despite the wealth of knowledge gained from studying hepadnaviruses in their natural hosts, the types of experiments that could be devised to elucidate the mechanisms of pathogenesis have been limited.

Hepaciviruses

The family Flaviviridae contains three genera. Hepaciviruses contain exclusively HCV. Flaviviruses contain a number of insect-borne agents, including yellow fever virus, dengue
The genome of hepatitis B virus (HBV). As it exists in the virion, the 3.2-kb-long genome of HBV is a partially double-stranded structure. The long or minus strand is uniform in length and has a nick at a unique site. The short or plus strand has a unique 5′ end but a variable 3′ end that is different in individual virus particles. The minus strand encodes all of the virus proteins from four overlapping open reading frames (ORFs). One ORF encodes the major surface antigen (HBsAg) polypeptide (226 amino acids long; sometimes called small S) and two larger but minor HBsAg polypeptides. Among the latter, one contains the preS2/S sequences (middle S) and the other contains preS1/preS2/S sequences (large S). All of these polypeptides are found in the envelope of the virus and in subviral particles. A second ORF encodes the major nucleocapsid polypeptide, which has HBV core antigen (HBcAg) activity. A proteolytic fragment of the core polypeptide is the hepatitis B e antigen (HBeAg), which is a soluble polypeptide in blood that is a surrogate marker of virus replication. A third ORF encodes the polymerase, which is responsible for virus replication. Minus strand synthesis begins at direct repeat 1 (DR1) and plus strand at DR2. A fourth ORF encodes hepatitis B x antigen, which regulates virus gene expression and replication and is involved in the development of hepatocellular carcinoma.
virus, as well as Japanese and tick-borne encephalitis viruses. Pestiviruses are exemplified by border disease and swine fever viruses, as well as the bovine viral diarrhea virus, which is used as a surrogate model for some aspects of HCV biology. HCV is related to these genera in terms of genome structure, the production of a polyprotein, the structure and function of the corresponding polypeptides, and their likely mode of replication (Chambers et al. 1990; Neyts et al. 1999) (Figure 4). Unlike HBV, there have been no reports of closely related HCV-like hepaciviruses that naturally infect wild animals or that can be used to experimentally infect laboratory animals. This reality restricts the available animal model systems that could be used to understand pathogenesis and to evaluate putative antiviral compounds or other novel therapeutic approaches.

**Mechanisms in the Pathogenesis of CLD: Cytopathic Effects and/or Immune-mediated Disease?**

Direct cytopathic effects (CPEs) occur when the action of virus gene products or virus replication is toxic to the infected cell, resulting in cellular damage, and sometimes cell death. With the exception of the CPE observed in cell lines or transgenic mice that constitutively overexpress HBV antigens (Chisari et al. 1989; Dunsford et al. 1990; Yoakum et al. 1983), there is little evidence to suggest that the pathogenesis of HBV infection is mediated by virus-induced CPE. In support of this idea, HBV carriers and transgenic mice with sustained, high levels of virus replication have no evidence of liver disease (Guidotti et al. 1995; Hoofnagle et al. 1987). In addition, tissue culture systems that replicate HBV do not develop CPE (Sells et al. 1987; Sureau et al. 1986).

Strong evidence exists that the pathogenesis of HBV infection is immune mediated. For example, liver and peripheral blood lymphocytes demonstrate proliferative and cytotoxic activities against HBV antigens (Feitelson 1996; Ferrari et al. 1987; Marinos et al. 1995). In addition, HBV-infected patients under immunosuppressive therapy often have widespread virus gene expression in the liver and high levels of virus in the serum without liver disease; however, they often develop severe liver disease when immunosuppressive therapy is terminated and antiviral immune responses reappear (Bianchi and Gudat 1979). Furthermore, hepatitis B surface antigen (HBsAg) or HBV transgenic mice adaptively transferred with virus-specific cytotoxic T lymphocytes (CTLs) have been shown to develop either acute or fulminant hepatitis (Ando et al. 1993; Moriyama et al. 1990), as in human infections (Figure 1). In addition, HBV transgenic severe combined immunodeficient mice adaptively transferred with normal, syngeneic splenocytes develop either acute or chronic hepatitis (Larkin et al. 1999). Moreover, strong and rapid cell-mediated immune responses to multiple HBV antigens are characteristic of acute infections that resolve, whereas weak immune responses to few HBV antigens are characteristic of infections that become chronic (Chisari and Ferrari 1995; Feitelson 1989, 1996) (Figure 1). Hence, the pathogenesis of HBV is immune mediated, although the virus antigens and corresponding immune responses responsible for acute versus chronic liver disease, and that permit the persistence of virus during chronic infections, have not been clearly identified.

Flaviviruses tend to mediate pathology by CPE (Chambers et al. 1990), although the case is not so clear cut with HCV. The finding of severe cholestatic hepatitis in a subset of HCV-infected liver transplant patients (Collier and Heathcote 1998), and that HCV-associated CLD appears to be more severe in human immunodeficiency virus-infected patients compared with those without dual infections (Thomas et al. 1996), suggest CPE. This is because both types of patients are immunosuppressed. On the cellular level, the rounding and shrinkage of hepatocytes, alterations in cellular membranes, and the development of pyknotic nuclei in HCV-infected livers are also consistent with CPE (Omata et al. 1981). However, lack of liver pathology and elevated liver enzymes (transaminases) in the blood of acutely infected chimpanzees during the incubation period of infection, and the persistence of virus in chimpanzees and patients in the absence of liver disease (Bradley 2000), suggest that HCV is not directly cytopathic. Moreover, the development of lymphoid aggregates and follicles in the liver, bile duct damage, and activated sinusoidal inflammatory cells (Bronkhorst and ten Kate 1998), suggest that the lesions associated with chronic HCV infection are mediated by immune responses against virus-infected hepatocytes. Additional work has shown that T helper cells and CTLs are important effector cells in the pathogenesis of HCV (Diepolder et al. 1998), further supporting the likelihood of immune-mediated pathogenesis. However, the balance of the data for HCV does not exclude that some aspects of pathogenesis could be due to CPE while other aspects may be immune mediated (Figure 2). These features are important not only for the design of relevant animal models but also for the selection of single and multiple therapeutic approaches that are likely to target the underlying causes of CLD.

**Transgenic Mice for HBV**

The development of transgenic mouse technology, which permitted insertion of any gene(s) of interest into fertilized mouse ova, made possible the construction of mice that expressed the inserted sequences so that the function and consequences of transgene expression could be studied in vivo (Palmiter and Brinster 1985). This development provided opportunities to create easily manipulated laboratory animal models of HBV and HCV gene expression, replication, and disease. In 1985, two research groups created transgenic mice that produced the HBV envelope or HBsAg particles (Babinet et al. 1985; Chisari et al. 1985) encoded by the HBV surface antigen gene (Figure 3). These subviral particles lacked viral DNA and had the same morphology, diameter, density, and polypeptide composition as HBsAg...
Figure 4 Diagram of hepatitis C virus (HCV) polyprotein (A), the names and sizes (in kDa) of the individual mature gene products (B), and the known or putative function of each (C). In (A), the core/E1, E1/E2, E2/p7, and p7/NS2 junctions are cleaved by a cellular signal peptidase (●) to yield the structural proteins of the virus. The NS2/NS3 protein then undergoes autocatalytic cleavage (◇), which releases the mature NS3 serine protease. The latter cleaves the remainder of the nonstructural (NS) polypeptides (◆), which then assemble into membrane-associated replication complexes, where NS5B then replicates the RNA strands of the virus. p, protein; gp, glycoprotein. Modified from Major ME, Feinstone SM. 1997. The molecular virology of hepatitis C. Hepatology 25:1527-1538.

particles from infected human serum samples. Moreover, mice were tolerant to HBsAg so that, despite consistently high levels of circulating HBsAg, no liver disease was detected. Independently derived lines of HBsAg producing transgenic mice revealed preferential expression in the liver and kidneys (Burk et al. 1988). Other lines of transgenic mice were made and shown to fully support HBV replication; in all cases, no liver disease was observed (Araki et al. 1989; Farza et al. 1988; Guidotti et al. 1995). This further suggested that HBV was not directly cytotoxic. However, overexpression of HBsAg polypeptides containing preS sequences (Figure 3) resulted in their retention and accumulation in the liver of transgenic mice (Chisari et al. 1986). As mice aged and HBsAg continued to accumulate, it became toxic to the hepatocytes, causing chronic liver injury and inflammation, regenerative hyperplasia, transcriptional deregulation, aneuploidy, and finally the appearance of HCC (Chisari et al. 1989; Dunsford et al. 1990). Although there is no evidence that the overexpression of HBsAg causes toxic liver injury in human carriers, these studies highlighted the importance of chronic liver cell injury and hepatocellular regeneration to the pathogenesis of HCC. In this sense, these mice provide an explanation for the epidemiological findings that the most important risk factors for HCC development are the HBsAg carrier state and the presence and progression of CLD (Beasley et al. 1981) (Figure 1). HCC also developed in transgenic mice with sustained high levels of hepatitis B x antigen (HBxAg) in the liver (Kim et al. 1991; Koike et al. 1994; Ueda et al. 1995) in the absence of inflammatory liver disease, but not in transgenic mice with low or undetectable levels of HBxAg (Lee et al. 1990; Reifenberg et al. 1997). These data suggest that HBxAg is oncogenic. Mechanistic studies have shown that HBxAg binds to and inactivates the tumor suppressor p53 (Ueda et al. 1995), which contributes importantly to stepwise carcinogenesis. In addition, HBxAg appears to stimulate the production of transformation growth factor beta 1 (Yoo et al. 1996), which may contribute to pathogenesis through the triggering of increased hepatocellular apoptosis and the promotion of fibrogenesis.

The findings that persistently high levels of HBsAg in human carriers and transgenic mice do not result in the development of liver disease (Hoofnagle et al. 1987), and that immunosuppression ameliorates chronic hepatitis (Cote et al. 1991), suggest that liver cell damage may be immune
mediated (Feitelson 1996). This is also implied by the finding that HBV replicates without triggering CPE in tissue culture cells (Sells et al. 1987; Sureau et al. 1986). To test whether liver disease is immune mediated, clones of HBsAg-specific CTLs were generated and then used for adoptive transfer into HBsAg transgenic mice. The results showed a clearance of HBsAg from blood and a spike of alanine transaminase within a few days after adoptive transfer. Liver cell injury was confirmed histologically and was major histocompatibility complex class I restricted (Moriyama et al. 1990), suggesting that the adoptive transfer of HBsAg-primed CTLs resulted in an immune-mediated bout of acute hepatitis. Liver disease was dependent on the production of interferon gamma (IFN-γ) by the CTL, the intrahepatic levels of HBsAg expression, and the number of HBsAg-positive hepatocytes within the liver. A more detailed characterization of events over time revealed that initially, injected CTLs were associated with an increase in apoptosis among scattered hepatocytes. This was followed by the recruitment of antigen-nonspecific inflammatory cells, and then by the development of necroinflammatory foci that extended beyond the region where CTLs were present (Chisari and Ferrari 1995). Among mice that secreted HBsAg into blood, the disease was transient, nonfatal, and destroyed no more than 5% of the hepatocytes. However, when adoptive transfer was performed in transgenic mice that retained HBsAg, the adoptively transferred CTLs were activated to produce IFN-γ, which in turn activated intrahepatic macrophages. These and other antigen-nonspecific cells that were recruited into the liver displayed a delayed type of hypersensitivity response that resulted in the rapid development of fulminant hepatitis and the death of many animals (Ando et al. 1993). Immune-mediated acute hepatitis was also independently observed in rats transfected with a replication-competent clone of HBV DNA. When parallel experiments were conducted in normal rats, HBV DNA appeared in serum within a few days, followed by clearance of virus, a transient elevation of alanine transaminase in blood, and histopathological evidence of acute hepatitis in the liver. When the same experiment was conducted in T cell-deficient nude rats, no clearance of virus or development of liver disease was observed, suggesting that T lymphocytes play a central role in liver cell injury and the clearance of HBV (Takahashi et al. 1995). These findings independently confirm the immune-mediated nature of fulminant and acute hepatitis (Figure 1) and provide information regarding the putative mechanisms of disease.

Although this work established models of acute and fulminant hepatitis, the major clinical problem resides among carriers with CLD (Figure 1). Accordingly, adoptive transfer experiments were carried out in HBsAg-overproducing transgenic mice that did not secrete antigen. To overcome tolerance to HBsAg, these mice were irradiated, thymectomized, and reconstituted with T cell-depleted bone marrow before adoptive transfer. The results showed that the inflammatory response set up after adoptive transfer accelerated hepatocellular turnover and the appearance of HCC (Nakamoto et al. 1998), suggesting that even the development of HCC has an immune-mediated component. However, there is little evidence to suggest that cell-mediated immune responses against HBsAg are central because it is not clear that HBsAg is an immunological target in chronic human infections. For example, only a small percentage of HBsAg carriers develop CLD and HCC. Furthermore, there is no evidence that HBsAg is overexpressed in chronically infected human livers to the levels observed in the HBsAg transgenic mice (Ray 1979). In addition, there is no correlation between intrahepatic HBsAg expression and inflammatory cells (Feitelson 1989). Hence, although immune-mediated liver disease contributes importantly to the development of HCC, these results do not reflect the range of immune responses that develop in chronically infected people who develop CLD and HCC.

There is increasing evidence from the HBsAg and HBV transgenic mouse systems that the clearance of virus gene expression and replication could be accomplished by noncytolytic mechanisms that involve the appropriately timed production of cytokines (Figure 1). Early observations showed that multiple HBsAg-specific CTL clones stimulated in vitro with plate-bound anti-CD3e monoclonal antibodies produced IFN-γ, tumor necrosis factor alpha (TNF-α), and to a lesser extent TNF-beta mRNAs in RNA protection assays (Ando et al. 1993; Guidotti et al. 1994a). The presence of these cytokine mRNAs correlated with the development of acute hepatitis after adoptive transfer, suggesting that they are made in vivo. Interestingly, the administration of monoclonal antibodies against IFN-γ or TNF-α before adoptive transfer largely prevented the CTL-mediated reduction in HBsAg expression, suggesting a cytokine-mediated noncytopathic inhibition of virus gene expression. These cytokines have also been shown to inhibit virus replication in transgenic mice (Guidotti et al. 1996b). Interestingly, virus clearance has been observed without extensive hepatocellular necrosis in WHV-infected animals even though the great majority of hepatocytes in the woodchuck liver become infected (Kajino et al. 1994). The latter study implies noncytolytic clearance of virus in a natural infection. Such clearance is consistent with the idea that there are not enough virus-specific CTL precursors in the body to mediate direct cytotoxicity with every infected hepatocyte and that cytokines are needed to amplify antigen specific responses. In addition, the fact that HBV clearance is associated with clinical recovery in people, and not death, also suggests a noncytolytic mechanism of immunologically mediated antiviral defense. In noncytolytic clearance, cytokines cause reduced steady state levels of most viral mRNAs among infected hepatocytes (Tsai et al. 1995) by triggering the binding of three cellular proteins to the viral RNAs, which then results in HBV RNA destabilization and degradation (Heise et al. 1999). In HBsAg transgenic mice, reduced levels of intrahepatic HBV RNA were observed after treatment with TNF-α (Gilles et al. 1992) or interleukin 2 (Guidotti et al. 1994b, Guilhot et al. 1993). Moreover, administration of interleukin-12, which induces T and natural killer cells to produce IFN-γ, inhibits virus replication and gene expres-
sion in transgenic mice that support virus production (Cavanaugh et al. 1997). Finally, when the latter mice were infected with lymphocytic choriomeningitis virus, the cytokines triggered by that virus activated macrophages in the liver (e.g., TNF-α and IFN-α/β) effectively suppressed HBV replication (Guidotti et al. 1996a). This result not only suggests that the extensive network of intrahepatic macrophages may play an important role in viral clearance, but also may partially explain the suppression of HBV replication in many HCV-coinfected patients (Liaw 1995).

The development of CLD in HBV carriers (Figure 1) is a major target for therapeutics, yet none of the HBV transgenic models available develop CLD because all transgenic mice are tolerant to the products of the transgene. In contrast, people who are acutely infected with HBV (or HCV) are immunologically naive to the virus. So the question becomes: Can one devise a transgenic system that supports virus replication but is not tolerant to the virus so that the development and progression of CLD can be studied? In a recent report (Larkin et al. 1999), transgenic mice supporting HBV replication were constructed using severe combined immunodeficient mice hosts that lacked mature T and B cells (Schuler et al. 1986). Because the T and B cell compartments account for the bulk of specific antiviral immunity, these transgenic mice were not tolerant to HBV. A single adoptive transfer of 10 million unprimed, syngeneic splenocytes resulted in the development of chronic hepatitis, whereas a similar transfer of 50 million cells resulted in a bout of acute, resolving hepatitis. Hepatitis was accompanied by mononuclear infiltrates resembling acute and chronic hepatitis in humans and with the clearance of virus gene expression and replicative forms from the liver as well as clearance of HBsAg and virus DNA from the blood. Although this model confirms the immune-mediated nature of HBV-associated acute and chronic liver disease, it will permit dissection of the immune components and virus antigen targets that contribute to pathogenesis.

Transgenic Mice for HCV

The narrow host range and lack of suitable tissue culture systems for HCV have provided significant barriers to studying the basic biology of host/virus interactions (Figure 2), including pathogenesis, and for testing putative antiviral compounds. As a consequence, a number of groups have embarked on the development of transgenic models of HCV gene expression and replication. In one study, transgenic mice that had been made using the HCV envelope 1 (E1) and 2 (E2) genes (Koike et al. 1995) expressed the HCV envelope proteins in many organs, including the liver, but did not develop any liver disease in mice up to 16 mo of age. These findings suggested that the envelope proteins of HCV were not cytopathic (Koike et al. 1995). However, these mice developed sialadenitis resembling Sjögren’s syndrome (Koike et al. 1997), an autoimmune disease affecting the salivary glands and associated with HCV infection in humans (Haddad et al. 1992). However, independent work revealed that transgenic mice expressing high levels of E2 developed no evidence of liver disease (Pasquinelli et al. 1997). Likewise, HCV core transgenic mice did not develop evidence of liver disease or HCC (Pasquinelli et al. 1997), suggesting that core was not directly cytopathic. Another transgenic mouse expressing E1, E2 plus core also showed no evidence of histopathology when evaluated at 6 mo of age (Kawamura et al. 1997). In contrast, a different lineage of transgenic mice making the envelope and core proteins of HCV developed spontaneous focal inflammation, hepatocellular necrosis and degeneration, and altered foci with mitotic figures by 10 mo of age, suggesting CPE (Honda et al. 1999). Alternatively, the independent production of core transgenic mice resulted in the development of steatosis (fatty liver) in mice older than 3 mo of age (Moriya et al. 1997). Steatosis is found in infected human liver and may reflect CPE. Surprisingly, when these mice were held beyond 16 mo of age, they developed adenomas containing fat droplets in the cytoplasm of hepatocytes, followed by the development of poorly differentiated HCC within the adenomatous nodules (Moriya et al. 1998). HCV core was detected in most adenomas and in HCCs in 25 to 30% of the animals from two separate lines of transgenic mice, suggesting that it is directly oncogenic. Although the differences between these and other core transgenic mice are not clear, the mice that develop liver pathology express sustained high levels of core protein. In the life cycle of HCV, core protein is found almost exclusively in the cytoplasm of infected cells. However, its location in the nuclei of hepatocytes in transgenic mice that develop tumors suggests that HCV core may act as a transcriptional regulator that promotes tumor development (Chang et al. 1998, Ray et al. 1995).

Attempts were made to put these observations into context with the construction of multiple transgenic lines that carried the full-length HCV cDNA and supported HCV replication (Matsuda et al. 1998). Although HCV mRNA and HCV core protein were detected in the liver of one line, the levels of expression were low. In addition, no consistent histological changes were observed in the liver, suggesting that HCV replication is not directly cytopathic in vivo. In this context, the question still remains as to whether the likely immune-mediated pathogenesis of HCV, as suggested by the clinical literature (Figure 2), can be reproduced in a transgenic model. To address this question, conditional expression of the HCV envelope and core genes was carried out in mice using the Cre/loxP system (Wakita et al. 1998). When transgene expression was turned on, the expression of both envelope and core proteins was detected in the liver within 1 wk after transgene activation. A transient influx of T cells into the liver was accompanied by a peak of transaminases. At that time, core was also detected in the serum, suggesting it was released by damaged hepatocytes. When transgene induction was repeated in T cell-depleted mice, there was normal histology and transaminase levels, suggesting the inflammatory response and hepatocellular damage were T cell mediated. By day 14 after transgene activation, core was...
cleared from serum and replaced by anti-core (Wakita et al. 1998). These observations provide strong evidence that the pathogenesis of HCV infection is immune mediated, which provides a starting point from which the host-virus relationship can be studied in more detail.

Other Rodent Models of HBV and HCV

HBV and HCV gene expression, replication, and liver disease have also been studied in a number of nontransgenic rodent systems. For example, HBsAg expression has been demonstrated in the liver of adult rats intrahepatically injected with liposomes that carry an expression plasmid encoding the preS plus S polypeptides (Figure 3) (Kato et al. 1991). Within 2 days of injection, HBsAg was detected in serum and preS antigen in the liver. Because these antigens became undetectable within a few days, animals were given injections again with liposomes containing recombinant plasmid on day 7. Animals seroconverted to anti-HBs and, by day 14, developed mononuclear cell infiltrates within the liver around regions of focal necrosis. Hence, this approach could be used to trigger a bout of acute hepatitis in rats. Using a similar approach, cationic liposomes were used to deliver full-length HCV cDNA under control of the beta-actin promoter (Takehara et al. 1995). Two days after intrabiliary administration of liposomes, full-length HCV RNA was detected in the liver by reverse transcriptase/polymerase chain reaction, and HCV core was observed by immunohistochemical staining in a few percent of scattered hepatocytes. Alternatively, an HCV core/E1 expression vector has been introduced into rats and mice as a conjugate that is recognized by the asialoglycoprotein receptor on hepatocytes (Yamamoto et al. 1995). After chloroquine treatment (to increase transfection efficiency) and intraportal vein injection, receptor-mediated uptake resulted in the accumulation of most radiolabeled expression plasmid within hepatocytes, indicating successful liver targeting. HCV core RNA was detected by reverse transcriptase/polymerase chain reaction, and core protein was observed by immunohistochemical staining. The presence of HCV core protein in a few percent of the hepatocytes is similar to what has been observed in some human infections. These findings suggest that the continued development of this approach may lead to the development of a model that will permit the dissection of host/virus interactions in more detail.

Immunodeficient mice have also been used for transplantation of normal or infected human hepatocytes with the intent of providing a fully permissive system in vivo both to study infection and to evaluate antiviral compounds. In early work, a trimera was made from beige/nude/X-linked immunodeficient (BNX) mice that were total body irradiated and repopulated with bone marrow cells from severe combined immunodeficient mice (Galun et al. 1995). HCV-infected liver fragments from infected patients were then transplanted under the kidney capsule. HCV RNA was detected in the sera of up to half of these mice for 10 to 50 days after transplantation of liver tissue by reverse transcriptase/polymerase chain reaction. Although this model clearly demonstrated that human hepatocytes and HCV production could be maintained in vivo, the short duration and low levels of viremia limit the application of this model. Independent efforts have shown that when human hepatocytes are mixed with Matrigel, which provides a three-dimensional matrix that supports hepatocellular survival, and the mixture is transplanted into nonobese diabetic/severe combined immunodeficient mice, long-term survival is observed (Ohashi et al. 2000). These cells were capable of being infected with HBV in vivo, as demonstrated by positive staining for core and surface antigens, and supported virus replication, as shown by the presence of viral replicative forms over several months. In an alternative approach, when human hepatocytes immortalized by simian virus 40 large T antigen were stably transfected with HBV DNA and then transplanted into the spleen of Rag-2 immunodeficient mice (which lacked both T and B cells), human cells migrated to and fully integrated into the mouse liver parenchyma. These cells remained nontumorigenic, survived for at least 5 mo in the mouse liver, and produced high levels of viral DNA and detectable HBsAg in blood during that time. This model has provided a larger window for antiviral drug screening than other models that rely on transplantation of human hepatoma cells (Condreay et al. 1994) or woodchuck hepatocytes (Petersen et al. 1998) into immunodeficient mice, in which the hosts are killed by the outgrowth of tumor. However, none of the hepatocellular transplantation models are constructed to permit the development of immune-mediated liver pathogenesis, which remains a significant limitation.

Conclusions

There is considerable evidence that the pathogenesis of HBV and HCV infections is immune mediated. The challenge has been to develop easily manipulated animal models to study these human virus infections and, in particular, to understand the pathogenesis of disease as well as development of HCC. Although progress has been made in this direction with the development of several transgenic mouse models as well as several nontransgenic systems, the generation of additional models that encompass a broader range of host-virus interactions is required, especially with regard to the pathogenesis of CLD. Such models should be very useful for understanding the mechanisms of pathogenesis and for evaluating new therapeutic approaches aimed at both the virus and associated chronic liver diseases.

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