Transgenic technology and the study of hepatitis viruses: A review of what we have learned

David R Milich PhD

David R Milich. Transgenic technology and the study of hepatitis viruses: A review of what we have learned. Can J Gastroenterol 2000;14(9):781-787. Because of the absence of inbred animal models susceptible to infection by the hepatitis B (HBV), C (HCV) and delta (HDV) viruses, and the inability to culture these viruses, a number of investigators have produced transgenic (Tg) mice that express one or all the viral genes. This review attempts to catalogue and characterize the Tg mice produced to date. The topics addressed are HBV, HCV and HDV gene expression and regulation; HBV replication models and factors that inhibit replication; HBV pathogenesis models; HBV tolerance and persistence models; modulation of the immune response to HBV proteins in Tg mice; T cell receptor Tg mice; and models of hepatocellular carcinoma.

Key Words: *Hepatitis B virus; Hepatitis C virus; Hepatitis delta virus; Transgenic mice*

Les techniques transgéniques et l'étude des virus de l'hépatite : une synthèse de nos connaissances

RÉSUMÉ : En l'absence de lignées pures de modèles animaux sensibles à une infection par le virus de l'hépatite B (VHB), C (VHC) et delta (VHD), et devant l'incapacité de cultiver ces virus, un certain nombre de chercheurs ont produit des souris transgéniques (Tg) qui expriment un ou l'ensemble des gènes viraux. La présente synthèse a pour objet de cataloguer et de caractériser les souris Tg qui ont été produites à date. Les sujets traités sont l'expression et la régulation des gènes du VHB, VHC et VHD ; les modèles de réplication du VHB et les facteurs qui inhibent sa réplication ; les modèles de la pathogenèse du VHB ; les modèles de persistance et de tolérance du VHB ; la modulation de la réponse immunitaire aux protéines du VHB dans les souris Tg ; les récepteurs des lymphocytes T des souris Tg ; et les modèles de carcinomes hépatocellulaires.

Prompted by the limited host range of the hepatitis B virus (HBV) (as well as hepatitis C virus [HCV] and hepatitis delta virus [HDV]) and by the lack of in vitro culture systems to propagate these viruses, a number of investigators have expressed single viral proteins, combinations of viral proteins and the complete genome (in the case of HBV) in transgenic (Tg) mice. It was anticipated that expression of viral proteins in inbred mice, which possess well characterized immune systems, would allow detailed studies of the immune response to viral proteins in vivo. Use of liver-specific promoter systems has allowed viral protein expression to be targeted to the physiologically relevant site – the hepatocyte. These Tg systems have yielded a number of interesting insights into the biology, immunology and pathogenic potential of these liver-tropic viruses.

EXPRESSION OF VIRAL PROTEINS IN TG MICE

The first studies using Tg mice focused on the expression of the HBV envelope proteins in Tg mice (Table 1). These early studies demonstrated that the envelope proteins could be expressed in the liver by using liver-specific promoters or the HBV endogenous promoters (1-6). Using the endogenous HBV promoters resulted in expression in other tissues in addition to the liver. It was also demonstrated that envelope gene expression is developmentally regulated (5), and is posi-

This mini-review was prepared from a presentation made at the World Congress of Gastroenterology, Vienna, Austria, September 6 to 11, 1998
Department of Molecular Biology Cal-2, The Scripps Research Institute, La Jolla, California, USA
Correspondence and reprints: Dr David R Milich, The Vaccine Research Institute of San Diego, 3030 Science Park Road, San Diego, California 92121, USA. Telephone 858-587-9505 ext 223, fax 858-587-9208, e-mail dmilich@vrisd.org
Received for publication June 7, 1999. Accepted June 14, 1999

TABLE 1
Gene expression and regulation in transgenic mice

	Reference(s)
Hepatitis B virus gene expression	
Envelope proteins	1–6
Nucleocapsid proteins	
Hepatitis B core antigen	9–12
Hepatitis B 'e' antigen	12–14
X protein	15–17
Hepatitis delta virus gene expression	18
Hepatitis C virus gene expression	
Structural proteins	19–24

Table 2
Model of hepatitis B virus (HBV) replication in transgenic mice

	Reference(s)
Expression of the entire HBV genome using endogenous promoters	3,4,25-27
Infectivity of transgenic-derived HBV	28
Inhibition of replication	
Hepatitis B surface antigen-specific cytotoxic T lymphocytes	34
Cytokines	34,55
Heterologous hepatic infections	56,57
Overexpression of the hepatitis B 'e' antigen	58

tively regulated by androgens and glucocorticoids (6). Tg expression of the middle and major envelope proteins led to the assembly and secretion of 22 nm spherical particles as occurs during a natural HBV infection (7,8). Inclusion of the gene encoding the large envelope protein (pre-S[1]-containing) in the transgene construct resulted in the assembly of long, branching filamentous hepatitis B surface antigen (HBsAg) particles (7). If the large envelope protein was overexpressed in relation to the middle and major proteins by the use of an exogenous promoter (ie, albumin promoter), the filamentous HBsAg particles became trapped in the endoplasmic reticulum (ER) and were not secreted by the cell. This secretion defect eventually leads to a dramatic expansion of the ER in the hepatocyte and severe liver injury (8). It has been suggested that such hepatocytes are analogous to 'ground glass' hepatocytes observed in the liver of chronically infected patients (8). However, the nature of a putative secretion defect in infected hepatocytes is not clear.

Both HBV nucleocapsid proteins – the particulate hepatitis B core antigen (HBcAg) and the nonparticulate hepatitis B 'e' antigen (HBeAg) – have been expressed in Tg mice (Table 1). HBeAg is efficiently secreted into the blood, and HBcAg accumulates in the nucleus of hepatocytes (9-14). Expression of high levels of HBcAg revealed that intact HBcAg particles cannot traverse the nuclear membrane in either direction (11). The nonstructural HBV X protein has also been expressed in Tg mice (15-17). The X protein displays transcriptional transactivation properties, and it has been suggested that expression of this protein in the liver may play a role in the induction of hepatocellular carcinoma (HCC), which is associated with chronic HBV infection. In support of this hypothesis, high level liver-specific expression of the HBV X protein has led to HCC in Tg mice (15,16). However, other investigators have not observed the induction of HCC in independently derived X gene Tg mice (17).

Simultaneous infection with HDV and HBV, which provides the viral envelope necessary for HDV, is often associated with severe liver disease. This may be due to the additive effects of the immune response to both infections or to direct cytotoxic effects of HDV proteins because HBV gene products are not directly cytopathic. Both the large and the small forms of the hepatitis delta antigen (nucleocapsid) have been expressed in Tg mice, and no biological or histopathological evidence of liver disease was observed, suggesting that neither antigen is directly cytopathic to the hepatocyte in vivo (18).

Several groups have expressed the structural proteins of HCV (ie, core, E1, E2) in Tg mice. Three groups reported that the HCV core protein (19), the core and E2 proteins (20), and the core, E1 and E2 proteins (21) expressed in the livers of Tg mice caused no histological or biochemical evidence of liver disease or HCC. In order to examine the immune response to HCV structural proteins, Wakita et al (22) used the Cre/loxP system to conditionally express the core, E1 and E2 proteins in Tg mice. Core proteins were detected in serum seven days after transgene activation, concurrent with increases in serum alanine aminotransferase levels. Anticore antibody appeared 14 days after activation of the transgene. Furthermore, depletion of CD4 and CD8 T cells normalized the alanine aminotransferase increases as well as the pathological changes in the liver, suggesting that the immune response rather than the HCV proteins themselves mediated liver injury. Another group reported that expression of the HCV core protein in the liver of two independent Tg lineages resulted in progressive hepatic steatosis (fatty change), which characterizes HCV infection (23). Furthermore, after the age of 16 months, mice of both lineages developed hepatic tumours (24).

In summary, a number of investigators have expressed the structural proteins of the HBV, HDV and HCV viruses in Tg mice, and these Tg systems have yielded a number of interesting results, detailed below. However, one important conclusion from these studies is that the viral proteins themselves are not directly cytopathic within the liver. The only exceptions to this conclusion are the overexpression of the HBV large pre-S(1)-containing protein leading to a secretion defect (8) and the expression of the HCV core protein causing hepatic steatosis (23). A secretory defect during natural HBV infection has not been identified, and the cytotoxic effect of HCV core antigen has not been duplicated in other Tg lineages.

HBV REPLICATION IN TG MICE

Several laboratories have used constructs containing the entire HBV genome and HBV-derived regulatory sequences to produce Tg mice capable of viral replication (Table 2). Interestingly, HBV replication occurred in the kidney as well as the liver, and the supercoiled form of HBV DNA (cccDNA) has not been observed in any lineage of Tg mouse (3,4,25-27). In several Tg lineages, high level replication is sustained in 20% to 30% of primarily centrilobular hepatocytes, although virtually all hepatocytes express nuclear HBcAg (27). This suggests that hepatocytes infected in a natural infection may not all be equally permissive for HBV replication. High level replication and HBV gene expression are not associated with liver pathology (3,4,25-27). One group has successfully infected chimpanzees with viral particles derived from Tg mice (28). This model of HBV replication has provided the opportunity to examine the influence of viral and host factors on HBV replication, pathogenesis and clearance, including the effects of the immune response. For example, a number of factors have been shown to be capable of inhibiting HBV replication in the Tg model (Table 2). These and other approaches are discussed in the following sections.

TG MODELS OF HBV PATHOGENESIS AND LIVER INJURY

Studies employing the adoptive transfer of $CD8^+$, HBsAg-specific cytotoxic T lymphocyte (CTL) into HBV envelope-expressing Tg mice have demonstrated that CTL can induce an acute necroinflammatory liver disease similar to that of natural acute HBV infection (Table 3). The investigators described a three-step process through which the liver disease progresses. The first step involves the attachment of the donor CTL to HBsAg-positive hepatocytes, which are triggered to undergo apoptosis. Thereafter, between 4 and 12 h after injection, the CTLs recruit host-derived antigen-nonspecific inflammatory cells (ie, polymorphonuclear cells) that amplify the effects of the CTL (step 2) (29,30). This process results in necroinflammatory foci in which hepatocellular necrosis extends well beyond the location of CTL, suggesting that most hepatocytes are killed by cells other than the donor CTL. In these studies, the liver injury in most Tg lineages is transient and is confined to no more than 5% of hepatocytes. However, in recipient Tg mice that overexpress and accumulate HBsAg filaments (see above), the disease process proceeds to step 3, in which approximately half of the mice die of liver failure within 24 to 72 h of CTL transfer (31). The investigators suggest that this process resembles the histopathological features of HBV-induced fulminant hepatitis in humans, characterized by widespread necrosis of HBsAg-laden hepatocytes and diffuse lymphomononuclear inflammatory cell infiltrate and Kupffer cell hyperplasia (31). However, this model does not explain what the trigger for progression to step 3 may be in the absence of overexpression of large envelope protein, which has not been described in natural infection.

This same group of investigators has developed a model of prolonged chronic immune-mediated (CTL) hepatitis in HBV envelope Tg mice (32). This was accomplished by transferring HBsAg-specific CTL into thymectomized, irradiated, bone marrow-reconstituted envelope-Tg recipients. In addition to chronic hepatitis, the Tg recipients eventually (17 months) developed HCC, suggesting that a prolonged inflammatory immune response to an HBV protein can cause liver cancer (32), consistent with a number of observations in chronically infected humans (33).

In addition to the direct hepatocyte injury triggered by HBsAg-specific CTL, a second noncytolytic mechanism has been described in which the cytokines secreted by the HBsAg-specific CTL profoundly suppress hepatocellular HBV gene expression and HBV replication in Tg mice (34,35). The cytokines responsible for these noncytolytic antiviral effects were CTL-derived interferon (IFN)-gamma and CTL-induced tumour necrosis factor (TNF)-alpha (34). The antiviral regulatory potential of inflammatory cytokines was confirmed by the fact that administration of recombinant TNF (36), interleukin (IL)-2 and to a lesser extent IFN and IFN (37) also inhibited HBV gene expression in HBV envelope Tg mice. Furthermore, these cytokine effects were mediated by a post-transcriptional mechanism involving the degradation of cytoplasmic HBV mRNA (38,39). Interestingly, systemic treatment of HBsAg-Tg mice in vivo with IFN did not affect HBV gene expression, whereas CTL-delivered IFN did (37). This suggested that systemic IFN may have multiple effects, some of which may be cross-regulatory in terms of inhibition of HBV gene expression. Using these Tg models, it has been shown that all of the viral gene products, including the viral replicative intermediates, are susceptible to the effects of these CTL-derived inflammatory cytokines, suggesting that HBV is exquisitely sensitive to this effect. Recently, the source of the inflammatory cytokines responsible for inhibition of HBV gene expression has been extended to include $CD4^+$ T helper (Th) 1 cells (40,41). Furthermore, it was demonstrated that the HBsAg-specific $CD4^+$ Th cells could be elicited by DNA immunization in HBsAg-Tg mice. We recently produced T cell receptor (TCR) Tg mice in which the majority of the $CD4^+$ Th

TABLE 3
Transgenic models of hepatitis B virus (HBV) pathogenesis and liver injury

	Reference(s)
Hepatitis B surface antigen (HBsAg)-specific CD8+ cytotoxic T lymphocytes (CTL) mediate liver injury	
Acute hepatitis	29,30
Fulminant hepatitis	31
Chronic hepatitis and hepatocellular carcinoma	32
HBsAg-specific CD8+ CTL suppress HBV gene expression	
Cytokine-mediated suppression	35–37
HBsAg-specific CD8+ CTL Inhibit HBV replication	34
HBsAg-specific CD4+ T helper cells suppress HBV gene expression	40,41
HBe/HBcAg-specific CD4+ T helper cells mediate liver injury	42

TABLE 4
Transgenic models of hepatitis B virus persistence and/or tolerance

	Reference(s)
Secreted hepatitis B 'e' antigen (HBeAg) induces CD4+ T cell tolerance in utero	13
Tolerance to the HBeAg is variable and major histocompatability complex dependent	45
Secreted HBeAg can delete HBe/HBcAg-specific T helper 1 cells in adult mice	50
HBeAg-specific CD4+ T helper 2 cells can survive in HBeAg+ transgenic mice	48
The nonsecreted HBcAg is variably tolerogenic in transgenic mice	9,10
Tolerance to the hepatitis B surface antigen can be broken in transgenic mice	46,47

HBcAg Hepatitis B core antigen

cells were specific for the HBe/HBcAgs of HBV. Adoptive transfer of $CD4^+$ from these TCR-Tg mice polarized toward the Th1 subset into HBe/HBcAg-expressing Tg recipients results in liver injury. The degree and kinetics of liver injury depend on the affinity and specificity of the transferred Th cells and whether the recipient expresses HBeAg or HBcAg (42).

TG MODELS OF PERSISTENCE AND/OR TOLERANCE

Infants born to HBeAg-positive HBV carrier mothers invariably become persistently infected. To investigate the role of immunological tolerance mechanisms in chronic infection of the newborn, HBeAg-expressing Tg mice were generated (13). HBeAg-Tg mice represent a model system to examine the consequences of in utero exposure to HBeAg on HBc/HBeAg-specific immune responses (Table 4). Characterization of tolerance in HBeAg-Tg mice and mice rendered neonatally tolerant indicated that T cells but not B cells were rendered tolerant by HBeAg present in the serum at a concentration of 10 to 100 ng/mL; that T cell tolerance elicited by HBeAg also extends to HBcAg-specific T cells; that Tg mice produced anti-HBc but not anti-HBe antibodies upon immunization; that the immunoglobulin (Ig) G but not the IgM anti-HBc response was diminished in HBeAg-Tg mice; and that the T-cell tolerance induced by a single neonatal exposure to HBeAg was reversible and persisted for 12 to 16 weeks (13).

Many characteristics of immune tolerance found in HBeAg-Tg mice parallel the long term immunological status of neonates born to HBeAg-positive HBV carrier mothers, suggesting that the aberrant immunological responses of neonates born to carrier mothers may result from in utero exposure to HBeAg, as occurs in the Tg model. In support of the possibility that maternal HBeAg may traverse the placenta, non-Tg littermates born to HBeAg-Tg mothers were tolerant to HBc/HBeAg (13). Furthermore, HBeAg has been detected in the neonatal cord serum of infants born to HBeAg-positive HBV carrier mothers (43,44).

Studies in HBeAg-Tg mice revealed that the level of Th cell tolerance is dependent on the major histocompatibility complex (MHC) background and the Th cell site recognized by the Tg murine strain. The Th cells of HBeAg-Tg mice on an $H\text{-}2^s$ background (residue 120-131-specific) are very susceptible to tolerance induction by the neoself HBeAg, whereas the Th cells of HBeAg-Tg mice on an $H\text{-}2^b$ background (residues 129-140-specific) are significantly less tolerant to the same serum levels of HBeAg (45). Incomplete Th cell tolerance may also explain the ability of HBsAg-Tg mice to generate anti-HBs antibodies after immunization with HBsAg particles (46,47). The HBeAg-Tg model has provided the opportunity to examine the immunoregulatory properties of circulating HBeAg. For example, because the Th cells of HBeAg-Tg mice on an $H\text{-}2^b$ background are not completely tolerized, a single injection of an HBeAg-derived Th cell site (peptide 129-140) leads to sufficient anti-HBe 'autoantibody' production to complex with and mask the detection of serum HBeAg. This system serves as a model of HBeAg/anti-HBe seroconversion. The finding of residual and functional HBeAg-specific Th cells in the periphery of HBeAg-Tg mice suggests the possibility that similar HBeAg-specific Th cells are present and can be activated in chronically infected HBV patients. Subsequent studies revealed that the HBeAg-specific Th cells that evade tolerance and mediate anti-HBe autoantibody production in HBeAg-Tg mice are significantly 'altered' by their coexistence with the circulating HBeAg. The HBeAg-self-reactive Th cells surviving in HBeAg-Tg mice exhibit a unique fine specificity that can be distinguished from the HBeAg-specific Th cell repertoire of non-Tg mice and are comprised predominantly of Th2-like cells (48). The preferential survival of HBeAg-specific Th cells of the Th2-type in HBeAg-Tg mice is of particular interest because of the suggestive serological evidence that an imbalance in HBe/HBcAg-specific Th1/Th2 cell function may contribute to the induction and/or maintenance of persistent HBV infection (49). Cumulatively, these data suggest that conservation of secretion of the HBeAg may be a viral strategy to guarantee persistence following vertical transmission of HBV, which is the major source of chronic infection in endemic areas.

Secretion of the HBeAg is also conserved in the avian hepadna viruses in which in utero tolerance mechanisms described previously are not relevant. Furthermore, adult infection with the HBeAg-negative mutant virus is often associated with a fulminant course of infection rather than the relatively benign acute course that characterizes most adult-onset infections with wild-type HBV. These observations suggest that the HBeAg may function to modulate the immune response during chronic HBV infection in the adult in addition to its effects on neonatal tolerance. Because the HBeAg is a secreted protein, its effects on the HBe/HBcAg-specific Th cell repertoire can be mediated within the thymus and/or in the peripheral lymphoid compartment. We are particularly interested in the effects of circulating HBeAg on Th cells in the periphery because of the possible implications for HBV infection in the adult. We, therefore,

bred HBeAg-Tg (H-2^b) mice with Fas-defective lpr/lpr mice (50). Thymic T cell deletion (ie, negative selection) is not Fas-mediated, whereas peripheral clonal deletion appears to be Fas-mediated and impaired in lpr/lpr mice. Therefore, the availability of HBeAg-Tg mice on Fas-expressing or Fas-deficient backgrounds enabled us to examine the ability of secretory HBeAg to deplete HBeAg-specific Th cells in the periphery and determine whether Th1 or Th2 cells were preferentially affected by this mechanism. The results of the study in HBeAg-Tg/lpr mice suggested that circulating HBeAg preferentially depletes HBeAg-specific Th1-like cells in the periphery via Fas-mediated apoptosis and that HBeAg-specific Th2-like cells survive this process to a greater degree. This study suggested a mechanism by which the HBeAg may maintain or induce chronicity even during an adult infection. The fact that HBeAg is secreted and widely disseminated coupled with its ability to deplete HBe/HBcAg-specific Th1-like cells and spare Th2-like cells, which are more resistant to peripheral depletion mechanisms (ie, Fas-mediated apoptosis), make it an ideal candidate to promote viral persistence (50).

To determine the tolerogenic potential of a nonsecreted intracellular form of the HBeAg, namely the HBcAg, Tg mice expressing the HBcAg in the liver were produced. Expression of the HBcAg at birth in mice of an H-2^s MHC background resulted in Th cell tolerance (10). In another HBcAg-expressing Tg system, the serum amyloid P promoter was used and HBcAg expression in the liver was delayed until three to four days after birth. In these HBcAg-Tg mice, spontaneous anti-HBc antibody production occurred, indicating a lack of Th cell tolerance (9). Therefore, the time of developmental expression of a transgene is a critical factor in determining the degree of self-tolerance.

MODULATION OF THE IMMUNE RESPONSE TO HBV PROTEINS IN TG MICE

As noted previously, tolerance to the HBeAg in Tg mice is MHC dependent. Tolerance to HBeAg is complete in HBeAg-expressing Tg mice on an H-2^s genetic background, and incomplete on HBeAg-Tg mice on an H-2^b background. Therefore, a population of functional 129-140 peptide-specific Th cells coexist with circulating HBeAg in B10-HBeAg-Tg mice. The Th cells that evade deletion in HBeAg-Tg mice are quiescent unless activated by a dose of the 129-140 peptide as low as 0.6 µg, which induces anti-HBe seroconversion (45). This model has been useful to test reagents that may modulate anti-HBe seroconversion (Table 5). Soluble CD 152 (cytotoxic T lymphocyte antigen-4) has been shown to suppress anti-HBe seroconversion in this model (51). Similarly, IL-12 suppresses anti-HBe 'autoantibody' production and skews the Th cells toward the Th1 subset (52). Finally, injection of an envelope (pre-S[2]) T cell site peptide that binds the same MHC class II molecule as 129-140 peptide inhibits anti-HBe seroconversion by competitively binding to IA^b and preventing the activation of 129-140 peptide-specific Th cells (45). This may have important implications during a natural HBV infection because envelope proteins circulate in tremendous excess of the HBeAg. Therefore, 'intraviral' protein competition for MHC binding sites may be a viral strategy to saturate MHC molecules and prevent less abundant, and possibly more relevant, T cell recognition sites from gaining access to antigen presentation. Seroconversion in HBcAg-Tg mice has been accomplished by transferring primed non-Tg $CD4^+$ T cells into HBcAg-Tg recipients (50).

TABLE 5
Modulation of the immune response to hepatitis B virus proteins in transgenic (Tg) mice

	Reference(s)
Seroconversion in hepatitis B 'e' antigen (HBeAg)-Tg mice is major histocompatability complex (MHC) dependent	45
Inhibition of seroconversion in HBeAg-Tg mice	
Blockade of B7 costimulation	51
MHC blockade with envelope T cell sites	45
Interleukin-12 (T helper 2 → T helper 1 switch)	52
Seroconversion in hepatitis B core antigen-Tg mice	9,50
Seroconversion in hepatitis B surface antigen-Tg mice	
Protein immunization	46,47
Recombinant vaccinia virus	47
DNA immunization	40
Non-Tg dendritic cells	53

Several groups have also induced seroconversion to the HBsAg in Tg mice. Immunization of HBsAg-Tg mice with HBsAg in adjuvant induced low level anti-HBs production, approximately 500-fold less than in non-Tg littermates (46,47). Similarly, multiple immunizations with an HBsAg-vaccinia recombinant virus elicited low level anti-HBs seroconversion (47). Induction of seroconversion to the HBeAg, the HBcAg or the HBsAg has not resulted in liver injury, indicating that antibodies specific for these viral antigens are not pathogenic. Recently, one group immunized HBsAg-Tg mice with a DNA construct coding for the HBsAg and reported anti-HBs seroconversion, $CD4^+$ Th cell priming and inhibition of HBsAg gene expression probably due to IFN production (40). Finally, one group has suggested that the lack of anti-HBs production by HBV chronic carriers and in HBsAg-Tg mice is due to defective function of antigen-presenting cells rather than immune tolerance (53). It was suggested that circulating HBsAg reduced MHC class II and B 7.2 (CD86) expression on dendritic cells in HBsAg-Tg mice. Treatment with IFN or replacement of Tg dendritic cells with dendritic cells from non-Tg mice allowed Th cells from HBsAg-Tg mice to mediate anti-HBs production in vitro.

HBV-SPECIFIC TCR-TG MICE

Another application of the Tg technology to the study of HBV is the development of TCR-Tg mice in which a majority of $CD4^+$ or $CD8^+$ T cells are specific for an HBV protein. These mice are a source of monoclonal CTL or Th cells that can be used, much like monoclonal antibodies, for a variety of in vitro studies (Table 6). Furthermore, the TCR-Tg mice can be crossed with HBV protein-expressing Tg mice to produce

TABLE 6
Hepatitis B virus (HBV)-specific T cell receptor (TCR)-transgenic (Tg) mice

Source of monoclonal, naive T cells
T cell activation
T cell specificity
T cell-antigen presenting cell interactions
T cell-B cell interactions
T helper 1/T helper 2 polarization
HBV-Tg × TCR-transgenic 'double Tg mice'
Tolerance (ie, clonal deletion) mechanisms
Pathogenesis

double Tg mice, which are useful for examining tolerance mechanisms (ie, clonal deletion) and pathogenesis in vivo.

We recently developed several lineages of TCR-Tg mice harbouring $CD4^+$ Th cells specific for the HBe/HBcAg. The TCR genes were derived from T cell hybridomas produced from either wild-type mice immunized with HBeAg or HBeAg-Tg mice. The $CD4^+$ Th cells present in the TCR-Tg lineages derived from wild-type mice were never exposed to the HBe/HBcAgs, are of relatively high affinity and represent naive populations similar to an HBe/HBcAg-specific repertoire during an acute infection. In contrast, the $CD4^+$ Th cells present in the TCR-Tg mice derived from HBeAg-Tg mice are HBe/HBcAg-specific populations that were selected in the presence of circulating HBeAg, are of relatively low affinity and may serve as a model for the $CD4^+$ repertoire during chronic infections. The acute-like TCR-Tg lineages differ markedly from the chronic-like TCR-Tg lineages with respect to affinity and kinetics of T cell activation; fine specificity for either HBeAg or HBcAg; in vitro and in vivo immune responsiveness; Th1/Th2 subset differentiation; clonal deletion as analyzed in double Tg mice; and the kinetics and severity of liver injury occurring after adoptive transfer into HBe and/or HBcAg-Tg recipients (42). It is anticipated that these TCR-Tg and TCR-Tg × HBe/HBcAg-double Tg systems will provide unique insights into the complex interactions between HBV and the host immune response.

TG MICE AS A MODEL FOR HCC

Several investigators have reported the occurrence of cancerous lesions in the livers of mice Tg for various viral proteins (Table 7). As stated earlier, Tg mice expressing large amounts of the HBV pre-S(1)-containing large envelope protein demonstrate an ER storage disorder, leading to massive hepatocyte death, causing a secondary inflammatory and regenerative response that eventually leads to HCC in mice strains that have a low incidence of spontaneous HCC (54). Although excess pre-S(1) protein is unlikely to accumulate in natural infection, a link between chronic liver injury and HCC has been demonstrated in a number of systems, including a recent Tg model of immune-mediated chronic hepatitis by these same investigators (32). High level expression of the HBV X protein can also lead to HCC in Tg mice (15,16), although other investigators have not observed the induction

TABLE 7
Transgenic mice as a model for hepatocellular carcinoma

	Reference(s)
Overexpression of the hepatitis B virus (HBV) pre-S(1) protein	54
High level expression of the HBV X protein	15,16
Long term chronic hepatitis	32
Hepatitis C virus core expression	24

of HCC in independently derived X gene-Tg mice (17). This discrepancy may relate to the level of expression or the genetic backgrounds of the mice. The Tg mice that developed HCC were produced on a CD1 background, which displays a high spontaneous rate of HCC. Finally, the development of HCC in two independent lineages of mice Tg for the HCV core gene was recently reported (24). These HCV core-Tg mice develop hepatic steatosis early in life and after the age of 16 months develop hepatic tumours that first appear as adenomas. Thereafter, more poorly differentiated HCC develops from within the adenomas. This closely resembles the histopathological characteristics of the early stage of HCC in patients infected with HCV. Although this observation has not been repeated in other Tg mice expressing the HCV core protein, these particular Tg mice may be useful in determining the molecular events in hepatocarcinogenesis associated with HCV infection.

SUMMARY

The advantages of Tg technology for the study of hepatitis virus biology and the immune response to viral proteins are obvious from the literature reviewed herein. However, the limitations of this technology are not as obvious and should also be appreciated. Until virus receptor-Tg mice are developed, these systems are not models of infection and, therefore, are somewhat limiting. Furthermore, very high level gene expression and/or inappropriate tissue expression can lead to artifacts that are not relevant to the natural infection. These limitations are inherent in 'reductionist' model systems but can be ameliorated by interpreting the results obtained in these Tg systems in the context of what is known about the natural infection. In this way, we can avoid making the Tg model a disease rather than a model for the disease.

REFERENCES

1. Chisari FV, Pinkert CA, Milich, et al. A transgenic mouse model of the chronic hepatitis B surface antigen carrier state. Science 1985;230:1157-60.
2. Babinet C, Farza H, Morello D, et al. Specific expression of hepatitis B surface antigen (HBsAg) in transgenic mice. Science 1985;230:1160-3.
3. Burk RD, DeLoia JA, El Awady MK, et al. Tissue preferential expression of the hepatitis B virus (HBV) surface antigen gene in two lines of HBV transgenic mice. J Virol 1988;62:649-54.
4. Araki K, Miyazaki J-I, Hino O, et al. Expression and replication of the hepatitis B virus genome in transgenic mice. Proc Natl Acad Sci USA 1989;86:207-11.
5. DeLoia JA, Burk RD, Gearhart JD. Developmental regulation of hepatitis B surface antigen expression in two lines of hepatitis B virus transgenic mice. J Virol 1989;63:4069-73.
6. Farza H, Salmon AM, Hadchouel M, et al. Hepatitis B surface antigen gene expression is regulated by sex steroids and glucocorticoids in transgenic mice. Proc Natl Acad Sci USA 1987;84:1187-91.
7. Chisari FV, Filippi P, McLachlan A, et al. Expression of hepatitis B virus

large envelope polypeptide inhibits hepatitis B surface antigen secretion in transgenic mice. J Virol 1986;60:880-7.
8. Chisari FV, Filippi P, Buras J, et al. Structural and pathological effects of synthesis of hepatitis B virus large envelope polypeptide in transgenic mice. Proc Natl Acad Sci USA 1987;84:6909-13.
9. Takashima H, Araki K, Miyazaki J, Yamamura K, Kimoto M. Characterization of T cell tolerance to hepatitis B virus (HBV) antigen in transgenic mice. Immunology 1992;75:398-405.
10. Milich DR, Jones JE, Highes JL, et al. Extrathymic expression of the intracellular hepatitis B core antigen results in T cell tolerance in transgenic mice. J Immunol 1994;152:455-66.
11. Guidotti LG, Martinez V, Loh YT, Rogler CE, Chisari FV. Hepatitis B virus nucleocapsid particles do not cross the hepatocyte nuclear membrane in transgenic mice. J Virol 1994;68:5469-75.
12. Reifenberg K, Lohler J, Pudollek H, et al. Long term expression of the hepatitis B virus core- e- and X-proteins does not cause organ pathology in transgenic mice. J Hepatol 1997;26:119-230.
13. Milich DR, Jones JE, Hughes JL, Price J, Raney AK, McLachlan A. Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? Proc Natl Acad Sci USA 1990;87:6599-603.
14. Guidotti LG, Matzke B, Pasquinelli C, Schoenberger JM, Rogler CE, Chisari FV. The hepatitis B virus (HBV) precore protein inhibits HBV replication in transgenic mice. J Virol 1996;70:7056-61.
15. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991;351:317-20.
16. Koike K, Moriya K, Iino S, et al. High level expression of hepatitis B virus HBx gene and hepatocarcinogenesis in transgenic mice. Hepatology 1994;19:810-9.
17. Lee TH, Finegold MJ, Shen RF, DeMayo JL, Woo SL, Butel JS. Hepatitis B virus transactivator X protein is not tumorigenic in transgenic mice. J Virol 1990;64:5939-47.
18. Guilhot S, Huang SN, Xia YP, La Monica N, Lai MM, Chisari FV. Expression of the hepatitis delta virus large and small antigens in transgenic mice. J Virol 1994;68:1052-8.
19. Matsuda J, Suzuki M, Nozaki C, et al. Transgenic mouse expressing a full-length hepatitis C virus cDNA. Jpn J Cancer Res 1998;89:150-8.
20. Pasquinelli C, Shoenberger JM, Chung J, et al. Hepatitis C virus core and E2 protein expression in transgenic mice. Hepatology 1997;25:719-27.
21. Kawamura T, Furusaka A, Koziel MJ, et al. Transgenic expression of hepatitis C virus structural proteins in the mouse. Hepatology 1997;25:1014-21.
22. Wakita T, Taya C, Katsume A, et al. Efficient conditional transgene expression in hepatitis C virus cDNA transgenic mice mediated by the Cre/lox P system. J Biol Chem 1998;273:9001-6.
23. Moriya K, Yotsuyanagi H, Shintani Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997;78:1527-31.
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;4:1065-7.
25. Farza H, Hadchouel M, Scotto J, Tiollais P, Babinet C, Pourcel C. Replication and gene expression of hepatitis B virus in a transgenic mouse that contains the complete viral genome. J Virol 1988;62:4144-52.
26. Choo KB, Liew LN, Chong KY, Lu RH, Cheng WT. Transgenome transcription and replication in the liver and extrahepatic tissues of a human hepatitis B virus transgenic mouse. Virology 1991;182:785-92.
27. Guidotti LG, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. J Virol 1995;69:6158-69.
28. Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999;284:825-9.
29. Moriyama T, Guilhot S, Klopchin K, et al. Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. Science 1990;248:361-4.
30. Ando K, Guidotti LG, Wirth S, et al. Class I restricted cytotoxic T lymphocytes are directly cytopathic for their target cells in vivo. J Immunol 1994;152:3245-53.
31. Ando K, Moriyama T, Guidotti LG, et al. Mechanisms of class I restricted immunopathology: a transgenic mouse model of fulminant hepatitis. J Exp Med 1993;178:1541-54.
32. Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. J Exp Med 1998;188:341-56.
33. Milich DR, Jones JE, Hughes JL, et al. HBV infection, the immune response and hepatocellular carcinoma. In: Chadwick DJ, ed. Vaccines Against Virally Induced Cancers. Chichester: John Wiley and Sons, 1994:113-31.
34. Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity 1996;4:25-36.
35. Guidotti LG, Ando K, Hobbs MV, et al. Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. Proc Natl Acad Sci USA 1994;91:3764-8.
36. Gilles PN, Fey G, Chisari FV. Tumor necrosis factor-alpha negatively regulates hepatitis B virus gene expression in transgenic mice. J Virol 1992;66:3955-60.
37. Guidotti LG, Guilhot S, Chisari FV. Interleukin 2 and interferon alpha/beta downregulate hepatitis B virus gene expression in vivo by tumor necrosis factor dependent and independent pathways. J Virol 1994;68:1265-70.
38. Guilhot S, Guidotti LG, Chisari FV. Interleukin-2 downregulates hepatitis B virus gene expression in transgenic mice by a post-transcriptional mechanism. J Virol 1993;67:7444-9.
39. Heise T, Guidotti LG, Cavanaugh VJ, Chisari FV. Hepatitis B virus RNA-binding proteins associated with cytokine-induced clearance of viral RNA from the liver of transgenic mice. J Virol 1999;73:474-81.
40. Mancini M, Hadchouel M, Davis HL, Whalen RG, Tiollais P, Michel ML. DNA-mediated immunization in a transgenic mouse model of the hepatitis B surface antigen chronic carrier state. Proc Natl Acad Sci USA 1996;93:12496-501.
41. Franco A, Guidotti LG, Hobbs MV, Pasquetto V, Chisari FV. Pathogenetic effector function of CD4-positive T helper 1 cells in hepatitis B virus transgenic mice. J Immunol 1997;159:2001-8.
42. Chen M, Sallberg M, Thung SN, Hughes J, Jones J, Milich DR. Nondeletional T-cell receptor transgenic mice: model for the CD4(+) T-cell repertoire in chronic hepatitis B virus infection. J Virol 2000;74:7587-99.
43. Arakawa K, Tsuda F, Takahashi K, et al. Maternofetal transmission of IgG-bound hepatitis B e antigen. Pediatr Res 1982;16:247-50.
44. Lee S-D, Lo K-J, Wu J-C, et al. Prevention of maternal infant hepatitis B virus transmission by immunization: The role of serum hepatitis B virus DNA. Hepatology 1986;6:369-73.
45. Milich DR, McLachlan A, Raney AK, et al. Autoantibody production in hepatitis B e antigen transgenic mice elicited with a self T cell peptide and inhibited with nonself peptides. Proc Natl Acad Sci USA 1991;88:4348-52.
46. Mancini M, Hadchouel M, Tiollais P, Pourcel C, Michel ML. Induction of anti-hepatitis B surface antigen (HBsAg) antibodies in HBsAg producing transgenic mice: A possible way of circumventing "Nonresponse" to HBsAg. J Med Virol 1993;39:67-74.
47. Wirth S, Guidotti LG, Ando K, Schlicht HJ, Chisari FV. Breaking tolerance leads to autoantibody production but not autoimmune liver disease in hepatitis B virus envelope transgenic mice. J Immunol 1995;154:2504-15.
48. Milich DR, Schodel F, Peterson DL, et al. Characterization of self-reactive T cells that evade tolerance in hepatitis B e antigen transgenic mice. Eur J Immunol 1995;25:1663-72.
49. Maruyama T, McLachlan A, Iino S, Koike K, Kurokawa K, Milich DR. The serology of chronic hepatitis B infection revisited. J Clin Invest 1993;91:2586-95.
50. Milich DR, Chen MK, Hughes JL, Jones JE. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: A mechanism for persistence. J Immunol 1998;160:2013-21.
51. Milich DR, Linsley PS, Hughes JL, Jones JE. Soluble CTLA-4 can suppress autoantibody production and elicit long term unresponsiveness in a novel transgenic model. J Immunol 1994;153:429-35.
52. Milich DR, Wolf SF, Hughes JL, Jones JE. Interleukin 12 suppresses autoantibody production by reversing helper T cell phenotype in hepatitis B e antigen transgenic mice. Proc Natl Acad Sci USA 1995;92:6847-51.
53. Kurose K, Akbar SM, Yamamoto K, Onji M. Production of antibody to hepatitis B surface antigen (anti-HBs) by murine hepatitis B virus carriers: neonatal tolerance versus antigen presentation by dendritic cells. Immunology 1997;92:494-500.
54. Chisari FV, Klopchin K, Moriyama T, et al. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell 1989;59:1145-56.
55. Cavanaugh VJ, Guidotti LG, Chisari FV. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. J Virol 1997;71:3236-43.
56. Guidotti LG, Borrow P, Hobbs MV, et al. Viral cross talk: intracellular inactivation of the hepatitis B virus during an unrelated viral infection of the liver. Proc Natl Acad Sci USA 1996;93:4589-94.
57. Cavanaugh VJ, Guidotti LG, Chisari FV. Inhibition of hepatitis B virus replication during adenovirus and cytomegalovirus infections in transgenic mice. J Virol 1998;72:2630-7.
58. Guidotti LG, Matzke B, Pasquinelli C, Schoenberger JM, Rogler CE, Chisari FV. The hepatitis B virus (HBV) precore protein inhibits HBV replication in transgenic mice. J Virol 1996;70:7056-61.