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TRANSGENIC APPROACH TO HEPATITIS B VIRUS LIVER DAMAGE

Moriyama T, Gulhot S, Klopchin K, Moss B, Pinkert CA, Palmiter RD, Brinster RL, et al. Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. *Science* 1990;248:361-64.

EDITOR'S ABSTRACT

This study investigates the role of the immune system in the pathogenesis of hepatic injury during hepatitis B virus infection. A transgenic mouse line containing the gene sequences for the hepatitis B virus envelope region (pre-S1, pre-S2, and HBs Ags) ligated to mouse albumin regulatory sequences was developed. Mice of this lineage did not contract spontaneous liver disease and were immunologically tolerant to pre-S and HBs Ags. Nontransgenic mice of the same lineage were immunized with a recombinant vaccinia virus containing the coding region for the HBs polypeptide. Spleen cells from immunized mice were transferred into the transgenic mice. The recipient transgenic mice exhibited prompt falls in serum HBsAg, ap-

pearance of serum anti-HBsAg and biphasic patterns of serum ALT elevation. The first rise in serum ALT (3 to 7 days after spleen cell transfer) appeared to be due to antibody-mediated cell injury because it could be reproduced by administration of serum from immunized, nontransgenic mice to transgenic mice. The second rise in ALT, at 14 to 21 days, occurred long after disappearance of serum HBsAg and was associated with histological evidence of liver cell necrosis and infiltration of the liver by lymphocytes. In other experiments, the pattern of delayed injury was reproduced by transfer of specific cytotoxic Lyt 2+ (CD8) T cells from immunized nontransgenic mice to transgenic mice, suggesting a role for cytotoxic T lymphocyte-mediated immune injury. Further studies using a cytotoxic T lymphocyte cell line specifically sensitized to HBsAg suggested that (a) cytotoxicity was specifically directed against cells that expressed a small region of the HBsAg; (b) cytotoxicity was major histocompatibility complex-restricted (i.e. cells expressing HBsAg but not identical major histocompatibility complex antigens were not killed); (c) cytotoxicity was enhanced if target cells were pretreated with γ -interferon, which presumably enhances expression of major histocompatibility complex antigens; and (d) cytotoxicity and liver cell injury could be reproduced *in vivo* by transfer of HBsAg-directed cytotoxic T cell lines back into transgenic mice (which express hepatitis B antigens). In summary, transgenic mice were raised that expressed HBV-encoded antigens at the hepatocyte surface in a form recognizable by both major histocompatibility complex class I-restricted cytotoxic T lymphocytes and envelope-specific antibodies. The resultant liver cell injury indicates that chronic HBV infection may be mediated by antigen-specific, cell-mediated responses. Finally, these studies also suggest a role (perhaps minor) for viral antibodies in hepatocyte injury.

COMMENTS

This study is the most recent in a series of important papers by Chisari and colleagues regarding mechanisms of HBV infection. It highlights the usefulness of transgenic mice in exploring mechanisms of disease that have remained undefined for lack of an appropriate experimental model—in this case, the relative roles of direct viral cytotoxicity and immune-mediated cell injury in determining liver damage during acute and chronic HBV infections. A body of indirect evidence suggests that an immune response to HBV has a dominant role, including observations such as the lack of hepatic injury in many individuals chronically infected with HBV and the effect of treatment with immunosuppressive agents on enhancing viral replication and suppressing hepatic injury.

The transgenic model permits the incorporation of DNA sequence coding for any given polypeptide into a mouse embryonic genome. If successful, the sequence is expressed and processed in the test mouse in a manner similar to that of the native polypeptide. A promoter is required for expression of any gene, and the transgenic technique allows investigators to use either the endogenous promoter of the gene under study or an exogenous promoter with desirable properties. For instance, this group of investigators previously had used the metallothionein promoter ligated to HBV genes, allowing them to induce gene expression in certain tissues at will by administering zinc orally. In this study, the albumin promoter was used, which directs tissue-specific gene expression in the liver, a pattern of expression more reflective of HBV infection in humans. The transgenic mouse is immunologically tolerant to the expressed polypeptide. This can be a drawback of the transgenic model because many pathological processes are mediated by the host immune system. Chisari and co-workers, however, have used this property to attempt to distinguish between effects of the immune system and direct cytotoxic effects of HBV peptides.

Chisari et al. first described transgenic mouse lines bearing the pre-S1, pre-S2, S and X coding regions in 1985 (1). They were able to demonstrate mRNA transcripts for HBV proteins in liver, kidney, testis, heart and brain. HBsAg was found in serum and in numerous tissues, whereas the pre-S antigen was also found in liver. These animals did not exhibit clinical or histological abnormalities, suggesting that HBsAg expression alone is not sufficient to cause injury. Immunization of these transgenic mice with human HBsAg failed to elicit an antibody response; as expected, these mice were immunologically tolerant. Chisari and his coworkers later extended these preliminary observations using an albumin promoter in conjunction with the HBV gene sequences that would make the envelope protein expression more liver specific (2, 3). Transgenic lineages that expressed various amounts of HBsAg mRNA were selected for observation. The relative amount of large envelope protein to major envelope protein expressed also varied among the different transgenic lineages under observation. Envelope proteins are produced in at least three different sizes and denoted the major (traditional HBsAg), middle (pre-S2 and HBsAg) and large (pre-S1, pre-S2 and HBsAg) envelope proteins. Synthesis of each of these three proteins is thought to be determined by separate promoters and start codons. During HBV infection in man, the large envelope protein is synthesized in relatively greater amounts in early viral replication and is required for packaging of infectious virions. During chronic HBV infection, liver cells synthesize mainly the major envelope protein, which forms the numerous 22-nm spheres of noninfectious HBsAg seen in large numbers in serum. By ligating a complete sequence for envelope protein, including the pre-S1 and pre-S2 regions to an albumin promoter, Chisari and his colleagues created a series of transgenic mice that

expressed all three envelope proteins (large, middle and major). When mice that expressed relatively large amounts of large envelope protein were examined for 4 mo to 2 yr, the investigators observed development of (a) intracellular accumulation of large envelope protein in distorted endoplasmic reticulum (ER); (b) decreased hepatic secretion of the major envelope protein; (c) hepatic inflammation with infiltration of mononuclear cells and polymorphonuclear leukocytes; (d) hepatocellular necrosis and regeneration; (e) increased serum ALT levels; and (f) tumor nodules resembling HCC (4). These observations are consistent with those of Persing, Varmus and Ganem (5) and others that suggest that synthesis of large envelope protein causes retention of HBsAg polypeptides in the ER, resulting in decreased hepatocyte secretion of these proteins. Chisari's findings suggest that hangup of large amounts of protein in ER is directly toxic to hepatocytes, leading to hepatocellular injury, inflammation and HCC. Thus these previous studies support a direct cytotoxic role for hepatitis B-mediated liver injury, at least in cases where the large envelope protein is produced in large amounts. Whether this mechanism for liver injury actually occurs in humans with chronic HBV infection is unknown, but the association of chronic liver injury with impaired secretion of HBsAg should be testable because specific antibodies and cDNA probes for HBsAg polypeptides are available.

In the study presented here, Chisari and colleagues reversed directions to use transgenic mice to test the alternate mechanism of hepatocellular injury—that of immune-mediated injury. An ideal model would be one that allows controlled introduction of individual components of the immune response. The authors accomplished this feat with impressive results. They used transgenic mice that expressed large and major proteins under direction of an albumin promoter; however, the level of polypeptide production was low and these immunotolerant mice did not suffer spontaneous liver injury. The investigators then reconstituted part of an immune response to HBsAg by transferring long-lived spleen cells from nontransgenic mice immunized to the major (HBsAg) envelope protein into the transgenic mice. Clearance of serum HBsAg, liver cell injury and hepatic inflammation developed in transgenic mice that received spleen cells from immunized mice but not from unimmunized mice. Early clearance of serum HBsAg and liver cell injury may have been due to antiviral antibodies, whereas a second phase of prolonged injury appeared to be due to cell-mediated hepatocyte injury from specific cytotoxic T lymphocytes. T cells only killed cells expressing both parts of the major envelope protein (HBsAg) and identical major histocompatibility complex (MHC) antigens, a pattern of classic MHC class I-restricted cytotoxic T lymphocyte killing in which T cells respond only when the specific antigen they are primed against is presented in conjunction with "self" recognition signals (MHC identical antigens). Cells that expressed HBsAg but not MHC antigens (such as

HBsAg-transfected mouse thymoma cells) were not killed. Hepatocyte killing was increased by treatment with γ -interferon, which is thought to increase expression of MHC antigens on hepatocytes. These observations suggest that immune-mediated cell killing may contribute greatly to cell injury in hepatitis B infection and supports clinical findings that modulation of the immune response affects liver injury.

How well does this remarkable animal model mirror the liver injury seen in humans with chronic hepatitis B? The answer is not known; however, observations from a number of investigators have shown the presence of numerous CD8⁺ T lymphocytes in areas of necrosis in infected human liver, some of which display signs of activation (6). Furthermore, human hepatocytes in areas of periportal necrosis display MHC class I and II antigens (7). Finally, some lymphocytes eluted from hepatitis B-infected human liver tissue recognize HBsAg (8) or HBeAg (9). These findings, therefore, demonstrate that at least the elements of specific hepatitis B-directed, cytotoxic T cell-mediated hepatocyte injury in humans are in place. These results also provide a rationale for the use of interferon or other immunomodulating agents in the treatment of chronic hepatitis B.

It will be interesting as well to see future studies from this laboratory that will undoubtedly explore the relationship between direct cytotoxic effects of hepatitis B and immune-mediated injury. These findings also open the way for further human studies to determine the basis for the varied response of humans to hepatitis B infection, ranging from resolved acute hepatitis B to chronic hepatitis B in healthy carriers to chronic active hepatitis B.

W. MICHAEL McDONNELL, M.D.
REBECCA W. VAN DYKE, M.D.
*Division of Gastroenterology
University of Michigan
Ann Arbor, Michigan 48109*

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DETECTION OF HEPATITIS C VIRAL RNA BY THE POLYMERASE CHAIN REACTION

Weiner AJ, Kuo G, Bradley DW, Bonino F, Saracco G, Lee C, Rosenblatt J, et al. Detection of hepatitis C viral sequences in non-A, non-B hepatitis. *Lancet* 1990;335:1-3.

Garson JA, Tedder RS, Briggs M, Tuke P, Glazebrook JA, Trute A, Parker D, et al. Detection of hepatitis C viral sequences in blood donations by "nested polymerase chain reaction" and prediction of infectivity. *Lancet* 1990;335:1419-1422.

EDITOR'S ABSTRACT

These studies used the polymerase chain reaction (PCR) to identify hepatitis C virus (HCV) RNA in clinical samples collected from patients with chronic liver disease or from blood donors. The relative role of this assay compared with detection of HCV antibodies in serum was evaluated. Weiner and colleagues found that 9 of 15 patients with non-A, non-B (NANB) chronic hepatitis and the only patient with cryptogenic cirrhosis had persistent antibodies to a nonstructural protein of HCV (C100-3). Seven of the 10 antibody-positive patients had HCV RNA detected by a highly sensitive PCR assay; two of the antibody-negative patients also had HCV RNA. The pattern of viral RNA and antibody was evaluated in one patient with acute posttransfusion NANB hepatitis (PTNANB) and in three chimpanzees with acute infection. Viral RNA was detected early after infection and, in each case, before the appearance of antibody. In one chimpanzee, viral RNA disappeared when antibody became detectable. The authors conclude that most patients with chronic NANB liver disease have HCV infection.

Garson and colleagues found that 6 of 1,100 donor blood units were repeatedly positive for antibodies to the C100-3 antigen (anti-C100-3). Only one of these six donor units was found to contain HCV RNA by a "nested PCR" assay. Nested PCR refers to the use of two successive rounds of amplification, with the second round using primers internal to, or nested within, the original two primers. The positive donor unit was the only one of the six antibody-positive units to cause posttransfusion hepatitis in a recipient. Sera from three other confirmed cases of posttransfusion hepatitis were evaluated and in each case one donor unit of blood was found to contain HCV RNA and anti-C100-3. The authors concluded that anti-C100-3 may not be a good predictor of infectivity in blood donors and that direct identification of viral RNA may be required.