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THE IMMUNE RESPONSE IN CHRONIC HEPATITIS B VIRUS INFECTION: THE "CORE" OF THE PROBLEM?

Ehata T, Omata M, Yokosuka O, Hosoda K, Ohto M. Variations in Codons 84-101 in the Core Nucleotide Sequence Correlate with Hepatocellular Injury in Chronic Hepatitis B Virus Infection. J Clin Invest 1992;89:332-338.

ABSTRACT

Individuals with chronic hepatitis B virus (HBV) infection are generally divided into asymptomatic healthy carriers and patients with chronic liver disease. Several studies have suggested that the hepatitis B core antigen could be an immunological target of cytotoxic T lymphocytes (CTL). To investigate the possible pressure site from CTL, the entire core region of HBV DNA was sequenced in 30 subjects (10 asymptomatic healthy carriers and 20 patients with chronic liver disease). No significant changes in the nucleotide sequence and deduced amino acid residue were noted in the 10 healthy carriers. In contrast, a cluster of changes in a small segment of 18 amino acids (codons 84-101 from the start of the core gene) was found in 15 of the 20 chronic liver disease patients. All these 15 patients had advanced liver diseases (chronic active hepatitis and cirrhosis), whereas only mild liver disease (chronic persistent hepatitis) was found in the five patients without mutations. These data suggest that the region with mutation clustering is the major target of CTL, and that the mutations evolve under the pressure of immune selection.

COMMENTS

Of the many patients infected with HBV, only approximately 10% progress to have chronic liver disease (1). Although the clinical and pathological stages in this progression are well documented, the mechanisms underlying the hepatocellular damage remain an enigma. These patients have evidence of chronic viral replication, as manifested by the presence of HBV DNA and HBeAg. However, the hepatic injury is not thought to be related to direct viral damage because HBV has not been demonstrated to be cytopathic. Instead, most attempts to explain the pathogenesis of the chronic liver disease have focused on host-related factors.

The immune response to HBV infection is often implicated in the development of liver disease, and the hepatic injury is thought to result from the cellular immune response to infected hepatocytes (2). To understand the basis of this hypothesis, one must understand the mechanisms of immune recognition involved in cellular cytotoxicity. In response to viral infections, the immune system generates viral antigen-specific cytotoxic T cells (3). The antigen receptor complex on these CD8 lymphocytes recognizes viral antigens as peptides bound to class I histocompatibility antigens on the surface of infected cells. The peptides are generated by digestion of viral antigens and can be as short as 12 to 14 amino acids in length. Once the antigen receptors identify the peptide/class I complex, the T cells bind to the virus-infected cells. This binding is stabilized through the interaction of adhesion molecules. The T cells then lyse the virus-infected target cell (4). This action is essential in clearing a viral infection because of the need of destroying the intracellular source of viral replication. Released virus particles can then be neutralized and cleared by the circulating antibody. Thus hepatocytes are sacrificed by the immune system to control HBV.

Several types of data support the central role that antiviral cytotoxic immune responses play in the normal immune clearance of HBV infection. Cellular immunity to HBcAg is seen in patients who clear the viral infection and become antigen negative (5). In contrast, the reactivation of viral replication is often seen in patients who are immunosuppressed or given immunosuppressive medications (1). Evolution of chronic hepatitis B viremia to a nonreplicative, asymptomatic carrier state is often accompanied by the development of immunity to HBeAg (5). Importantly, whereas therapy with interferon may be effective in inhibiting HBV replication, it also augments antiviral cytotoxic immune responses in several ways (6). This latter activity may be most important to interferon's effectiveness in inducing viral clearance because agents that simply inhibit viral replication are rarely effective (7).

If immunity is of benefit in resolving acute hepatitis B infection, how might it be deleterious in patients with chronic hepatitis B infection? A scenario can be envisioned where ongoing viral replication exists that for some reason cannot be controlled or eliminated. Cellular immune reactions continue to destroy infected cells; however, because the virus is not eliminated, this leads to progressive hepatic destruction. Such a scenario would require a paradoxical immune response: effective cytotoxic activity that can lyse infected hepatocytes, but the overall immune response that is, for some reason, unable to clear the infection. Although virulence characteristics of a particular strain of hepatitis B could be

implicated as causing an infection that cannot be cleared, they do not explain why only some patients with evidence of chronic viral replication do not have liver disease develop. Complicating this further, most chronic hepatitis patients do not have evidence of immune abnormalities (1). This indicates that no identifiable immune defect characterizes either patients with chronic liver disease or the asymptomatic carrier state. Thus the exact interaction between chronic hepatitis B infection and the immune system that leads to liver disease is not clear.

In an attempt to clarify these issues, Ehata and his colleagues examined viral antigens from chronic hepatitis B patients who had liver disease develop. They reasoned that chronic immune "pressure" from ongoing CD8 T-cell cytotoxicity might select mutations in the antigenic portions of the virus. Viral mutations developing under pressure by immune surveillance have been reported in other viral infections, such as human immunodeficiency virus, where they give rise to virus variants that escape immune clearance (8). A similar event in hepatitis B might be related to the development of chronic infection.

The authors compared 10 asymptomatic carriers with 20 patients demonstrating liver disease who were all infected with the same subtype (adr) of hepatitis B. They examined the amino acid sequences of the antigens produced by HBV isolated from each of the patients and focused on the core antigen because it has been identified as a target of cellular immunity in patients with chronic infection. Using polymerase chain reaction, they were able to amplify out the coding sequence from the C gene of the virus and perform oligonucleotide sequence analysis. From this information, they deduced the amino acid sequence of the core antigen from each patient's viral isolate.

The result of this analysis was remarkable. Missense mutations were only present in the core antigens from patients with chronic liver disease. The core antigen amino acid sequences in the viruses isolated from the asymptomatic carriers were all identical with the consensus sequence reported for the adr virus subtype. In the patients with liver disease, however, very frequent amino acid substitutions were seen. Fifteen of the 20 liver disease patients had amino acid substitutions in their core antigen. Importantly, an 18 amino acid fragment was seen that contained most of the substitutions in these patients. A single substitution, leucine for isoleucine at position 97, was found in 9 of 20 patients. The authors were also able to observe one patient serially and identified that the mutation in the gene coding for the core protein occurred after the development of cytotoxic immune damage (as evidenced by elevated serum ALT levels). This suggested that the mutations seen in these patients were *de novo* and resulted from immune pressure on the virus. Finally, the mutations in the core protein were found to precede another described mutation in the hepatitis B genome, one that causes a stop codon in the precore gene preventing the production of the HBeAg. This seems to suggest that HBV, under pressure from the immune system, can continue to mutate until the virus is modified to prevent the production of antigenic parts of the molecule.

These findings have immediate implications for the care of patients with chronic hepatitis B infection. Screening for mutations in the core antigen may provide a marker for patients destined to have liver disease develop. This would help guide therapy with interferon or antiviral drugs. The ability to predict the development of liver disease also might obviate the need for liver biopsy in some patients. For these reasons alone, the findings in this study are important.

The more intriguing question is how these findings might help solve the riddle of why these patients have liver disease develop. These results fundamentally alter the concept of chronic hepatitis B infection. HBV can no longer be viewed as a passive entity in chronic infection. However, the exact role of the viral mutations these authors have observed is not obvious. The authors argue that the size of the area where most of the mutations occur is compatible with a T-cell epitope. This is possible, but this type of area in an antigen might also be compatible with an antibody epitope. In either case, if this area is a focus of immunological attack in the core antigen, it is possible that the mutations could lead to a virus that cannot be immunologically cleared. However, it is unlikely that this is the primary event in the induction of hepatocellular disease because evidence of liver damage precedes the development of the mutations.

Another interpretation of this report is that the patients with liver disease have an abnormal immune response that causes hepatocellular damage and that this immune response also leads to core antigen mutations. Fortuitously, the results from this article may allow the examination of the immune response. With the localization of the immunogenic portions of the core antigen, synthetic peptides or recombinant fusion proteins can be produced that correspond to the amino acid sequences of the normal and mutated antigens. Cytotoxic and proliferative T-cell responses to the normal and mutated core antigen may identify immune abnormalities in patients who have liver disease. This could reveal the actual immune processes involved in what has become one of the most common causes of chronic liver failure.

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AN "IRONIC" CASE OF MISTAKEN IDENTITY?

Adams PC, Ghent CN, Grant DR, Frei JV, Wall WJ. Transplantation of a donor liver with haemochromatosis: evidence against an inherited intrahepatic defect. Gut 1991;32:1082-1083.

ABSTRACT

An iron loaded liver from a 40 year old man with occult haemochromatosis was transplanted into a 19 year old woman with acute liver failure secondary to a paracetamol overdose. Increased parenchymal hepatic iron was found in a liver specimen at biopsy undertaken because of mild rejection 30 days after transplantation. After transplantation the patient had two episodes of liver rejection confirmed by biopsy. The hepatic iron concentration fell from 161 μmol/g on day 30 after transplant to 26.5 μmol/g (normal < 40) on day 210. Iron absorption, measured 45 days after transplant, was in the normal range at 12.4%. The rapid fall in hepatic iron and the normal iron absorption study result suggest that the genetic defect of haemochromatosis is not exclusively an intrahepatic defect.

COMMENTS

General agreement exists that cirrhosis and HCC in hereditary hemochromatosis (HHC) are secondary to excess iron deposition in the liver. Until recently, however, controversy in the literature has existed regarding the mechanisms of iron overload in this disease. Some have argued that the underlying defect in body iron balance lies in the liver, whereas others have pointed to the gut as the source of the problem, offering evidence that increased absorption of iron by the duodenal mucosa is the cause of iron overload in HHC.

Proponents of the hypothesis that iron overload in hemochromatosis is due to a hepatic defect have emphasized the unique pattern of hepatic iron deposition in this disorder. Hepatic iron accumulation in HHC patients is primarily parenchymal, with minimal deposition in reticular endothelial (RE) cells, at least in the early stages of the disease (1). As discussed recently by Bacon (2), this distinctive pattern of hepatic iron distribution has led to the controversial proposition that an intrinsic alteration of iron uptake, storage or transport by the RE cells in the liver of patients with HHC exists. An earlier study by Adams and coworkers (3) suggested that the level and pattern of hepatic iron deposition might influence iron absorption in rats. Six iron-overloaded livers from rats were transplanted into normal animals, and three normal livers were transplanted into iron-loaded animals. Iron loading was achieved through oral and parenteral administration, and total body iron counting with ^{59}Fe was used to measure iron absorption. Oral iron loading with carbonyl iron caused predominantly parenchymal iron deposition, whereas parenteral iron dextran administration resulted in RE cell iron deposition. Transplantation of livers with parenchymal iron overload into normal rats resulted in decreased iron absorption; however, transplantation of livers with RE cell iron overload had no effect on absorption as determined by this method. These results suggested not only that hepatic iron stores can influence iron absorption but also that the specific pattern of iron deposition within the liver can affect iron absorption. However, recent work in humans suggests that transferrin receptor expression in the hepatocyte plasma cell membrane is reduced in hemochromatosis patients just as in patients with secondary iron overload (4). Furthermore, hepatic transferrin receptor is expressed inversely to the level of hepatic iron stores in patients with HHC (5). Pietrangelo et al. (6) subsequently have directly confirmed that hepatic transferrin receptor messenger RNA (mRNA) content is depressed in HHC (6). These studies demonstrated that transferrin receptor expression in the livers of HHC patients is appropriately decreased, arguing against the hypothesis that a primary disorder of hepatic transferrin-iron transport in HHC exists.

A number of recent articles have examined iron absorption and transmucosal transfer in the proximal small intestine in patients with HHC. Immunohistochemical techniques have shown that duodenal mucosal content of ferritin in HHC patients is significantly lower than in controls or patients with secondary iron overload (7). At the molecular level, appropriate downregulation of transferrin receptor mRNA in the duodenum of patients with secondary iron overload is seen, which is accompanied by increased quantities of ferritin mRNA (8). In contrast, patients with HHC have high levels of transferrin receptor mRNA and low levels of mRNA for both ferritin H and L subunits. Kinetic studies of iron absorption in HHC patients using orally administered radiolabeled iron have been performed; analysis of rate constants for mucosal uptake and transmucosal transfer show no increase in mucosal uptake but rather accelerated transfer of iron from the mucosa to blood (9). The exact cause for this increased transfer of iron from the mucosa to blood in HHC patients is unknown, although it is tempting to speculate that the failure of mucosal ferritin expression could remove the "gatekeeper" function of this iron storage protein.

Two recent case reports serve nicely to illustrate the pathophysiological condition of iron metabolism in patients with HHC. In the article under discussion, Adams et al. report that a hemochromatotic liver was inadvertently transplanted into a patient with fulminant liver failure caused by acetaminophen. A percu-