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 HBcAg (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.

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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.

COMMENTS

These studies underscore the usefulness of a powerful research tool, the PCR, to address clinically important questions concerning the biology of hepatitis viruses. With this method one can definitively identify viral nucleic acid in clinical samples and, therefore, better define the clinical course of infection and the meaning of HCV antibodies. Our comments will address PCR methodology and the clinical usefulness of these authors' observations.

Interpretation of PCR data in clinical studies requires consideration of methodological issues. PCR entails the use of oligonucleotide primers, which are homologous to known nucleic acid sequences to enzymatically amplify the DNA between the two primers. The method is sensitive enough to amplify a single DNA molecule to levels that are easily detected. As used in these studies, the method can be modified to amplify RNA sequences by first synthesizing complementary DNA (cDNA) with the enzyme reverse transcriptase.

Use of PCR can generate false-positive and false-negative results. Much attention has been devoted to the concern over false-positive results when using PCR assays. Contaminated assay solutions or "carryover" from pipettes can result in identification of a DNA fragment in true negative samples. As used in these two studies, strict adherence to published laboratory precautions (2) and the inclusion of negative controls for each assay reduce the likelihood of false-positive results.

Because of the exquisite sensitivity of PCR, the possibility of false-negative results is often overlooked. False-negative results may be more likely when RNA is amplified because of the need for cDNA synthesis before PCR. Therefore, when the sensitivity of PCR assays is evaluated, the reverse transcription reaction must be included in the assessment. Weiner et al. were able to detect 3 CID (chimpanzee infectious doses)/ml with their assay. These investigators synthesized cDNA from chimpanzee serum and amplified the cDNA with oligonucleotide primers; the PCR products were probed with a radiolabeled oligonucleotide, which greatly increases the sensitivity for detection of amplified DNA. In contrast, Garson used a partial clone of HCV (ds DNA) to assess sensitivity and, therefore, did not include the cDNA-synthesis step. Despite this caveat, Garson's use of a second round of amplification using nested primers achieved a level of sensitivity similar to that of the study by Weiner without using a radioactive probe; this represents a significant simplification of the method and makes it potentially more amenable for use in clinical laboratories.

An additional variable is the amount of serum or tissue used to extract nucleic acids for use in the assay. In the study by Garson, as little as 5 µl serum was used to extract nucleic acids, and only 20% of this was used in the reverse transcriptase reaction. If the average number of virions in infected serum is 10^2 to 10^4/ml as suggested by Weiner, there may be <1 virion/5 µl plasma.

Perhaps the most important consideration for false-negative results is the existence of different strains or subtypes of HCV. In Japan, at least two strains of HCV have been identified (3). These two strains, termed HCV-K1 and HCV-K2, have 80% and 67% homology, respectively, with the sequence of the original isolate over the limited area of reported sequence (4). Significant mismatches between PCR primers and viral sequence could markedly diminish the sensitivity of the assay or completely eliminate amplification. Furthermore, significant nucleotide or amino acid sequence differences in one portion of the virus may not affect antibody recognition of C100-3 protein (2). Therefore, HCV strains in various populations must be sequenced and primers carefully chosen to amplify prevalent strains in the population under study. This is a potential reason for the low detection rate of HCV RNA in the study by Garson, although the lack of infectivity of anti-HCV–positive, HCV RNA–negative blood argues against this possibility.

These studies assist the practicing physician in interpreting anti-HCV serological results (using anti–C100-3 tests) in two important clinical situations. The first concerns patients with acute or chronic PTNANB hepatitis; the second concerns persons found to have anti–C100-3 without evidence of liver injury. In both circumstances the crucial question is whether the person has HCV RNA. Weiner's study indicates that the presence of anti–C100-3 in persons with chronic PTNANB hepatitis is frequently accompanied by HCV RNA, indicating that the patient is infectious. Furthermore, these data hint that viral replication is a necessary factor to sustain hepatic injury in chronic PTNANB hepatitis. Similarly, the data from one chimpanzee and one surgical patient, which show that acute HCV hepatitis is accompanied by high levels of HCV RNA, suggests that viral replication is involved in the mechanism for hepatic injury in acute PTNANB hepatitis also.

A crucial observation of these authors is that HCV RNA may be present in acute or chronic episodes of PTNANB hepatitis when anti–C100-3 is not detectable. This may not represent a false-negative result so much as the temporal pattern for development of this particular antibody. Moreover, previous studies by Alter et al. (5) would suggest that some (2 of 5) patients with acute PTNANB hepatitis do not proceed to anti–C100-3 positivity. In that study, HCV RNA was not measured, so the conclusion that these anti–C100-3–negative acute PTNANB hepatitides were indeed HCV-induced remains presumptive. Nonetheless, taken together one can conclude that the absence of anti–C100-3 in a clinical setting suggestive of HCV infection must not be taken to indicate that HCV has been excluded from the differential diagnosis. Put more simply, HCV infection may be present when currently available anti-HCV tests are negative.

What is the significance of positive results for anti–C100-3 in the absence of overt liver dysfunction? Garson et al. address this important question. We assume (although it is not stated) that all blood for transfusion in their study was screened for elevation of serum transaminases and HBc antibody. Their data show that

these blood donors frequently do not harbor HCV RNA and are not infectious. This is borne out by the absence of PTNANB hepatitis in the five recipients of anti–C100-3 HCV RNA–negative blood, whereas the only recipient of anti–C100-3–positive, HCV RNA–positive blood did contract PTNANB hepatitis. It would be of interest to know the subsequent clinical course of the donor of the latter blood. At present it is unclear whether HCV infection can occur without hepatitis, either acutely or chronically. If chronically infected, this donor could be analogous to a benign chronic carrier of HBsAg. Conversely, in view of Weiner's data, one may question why this donor did not have hepatitis if viral replication and hepatitis appear to go hand in hand. Clearly there is much to be learned about the mechanisms of cellular injury in HCV infection.

Garson's study does not indicate whether there were any anti-HCV–negative cases of PTNANB hepatitis among the recipients of the 1,100 units of blood tested. In a previous study by Van der Poel et al. (6), of 5,150 units of blood transfused into 383 recipients, 6 of 34 (18%) recipients of anti–C100-3–positive blood contracted NANB hepatitis. This result is similar to that of Garson — one of six recipients of anti–C100-3–positive blood. Interestingly, in Van der Poel's study, 3 of 349 recipients of anti–C100-3–negative blood contracted NANB hepatitis. In contrast, Esteban et al. (7) have reported on 280 transfusion recipients of 1,109 units of blood among whom 27 (9.6%) contracted PTNANB hepatitis. In this study anti-HCV status was assessed by anti–C-100 ELISA and also by the more recently developed recombinant immunoblot assay, which recognizes antibodies to two HCV epitopes, C-100 and 5.1.1. HCV RNA was not measured. Sixteen of the 27 PTNANB patients in Esteban's study received anti-HCV–positive blood, whereas 11 received anti-HCV–negative blood. However, 24 of 27 patients with PTNANB hepatitis became anti-HCV–positive during 52 wk of follow-up. Only two recipients of anti-HCV–positive blood did not contract PTNANB hepatitis. It is not clear why there was so marked a disparity in infectious potential of anti-HCV–positive blood between the studies by Garson and Van Der Poel and that of Esteban (18% vs. 88%). However, in agreement with Weiner's data, one can infer from Esteban's study that infectious HCV may be present in blood without detectable anti-HCV. The application of PCR technology to identification of HCV RNA in potentially infected sera is one possible solution to this clinical conundrum.

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NEW INSIGHTS INTO CANALICULAR ORGANIC ANION SECRETION

Oude Elferink RPJ, Ottenhoff R, Liefting WGM, Schoemaker B, Groen AK, Jansen PLM. ATP-dependent efflux of GSSG and GS-conjugate from isolated rat hepatocytes. Am J Physiol 1990;258:G699-G706.

EDITOR'S ABSTRACT

The effect of partial and complete dissipation of the membrane potential and partial depletion of cellular ATP content on the efflux of dinitrophenyl-glutathione and oxidized glutathione was examined in hepatocytes isolated from normal and mutant (TR⁻) rats exhibiting defective organic anion transport. Whereas alterations in the membrane potential difference had no effect on the initial efflux rate of dinitrophenyl-glutathione and oxidized glutathione, depletion of cellular ATP inhibited dinitrophenyl-glutathione and oxidized glutathione efflux and a linear relationship between the cellular ATP content and the initial efflux rate of dinitrophenyl-glutathione was observed in normal isolated rat hepatocytes. In contrast, depletion of cellular ATP content had no significant effect on the slower rate of dinitrophenyl-glutathione efflux from TR⁻ rat hepatocytes. These findings implicate an ATP-dependent hepatic transport system for oxidized glutathione and glutathione conjugates that is absent in TR⁻ mutants.

Kitamura T, Jansen P, Hardenbrook, Kamimoto Y, Gatmaitan Z, Arias IM. Defective ATP-dependent bile canalicular transport of organic anions in mutant (TR) rats with conjugated hyperbilirubinemia. Proc Natl Acad Sci USA 1990;87:3557-3561.

EDITOR'S ABSTRACT

Fluorescence image analysis reveals normal secretion of a fluorescent bile acid (fluorescein isothiocyanate glycocholate into the canalicular lumen of isolated normal and TR⁻ mutant rat hepatocyte couplets, but negligible canalicular accumulation of a non-bile-acid organic anion (carboxydichlorofluorescein diacetate) in TR⁻ hepatocyte couplets. Canalicular mem-