Welcome to issue 30 of Hepatitis Research Review.

It seems that co-infection with hepatitis E virus (HEV) is uncommon in HIV+ adults, at least according to research conducted in the USA. Thus, widespread screening for HEV in HIV-infected persons in the USA may not be necessary. The limitation of currently available HEV infection models is that they largely rely on hepatoma cells, which are unable to fully support HEV infection and replication. Now, researchers from Belgium have developed a physiologically relevant model system for studying the biology of HEV replication and strategies to inhibit viral replication. This model provides a useful alternative for hepatoma cell lines and human primary hepatocytes in the study of the viral biology of the HEV.

I hope you enjoy the selection in this issue and I welcome your comments and feedback.

Kind Regards,
Professor Stephen Riordan
stephen.riordan@researchreview.com.au

Acute and chronic hepatitis E virus infection in human immunodeficiency virus-infected U.S. women

Authors: Kuniholm MH et al.

Summary: These researchers used a high-throughput nucleic acid testing (NAT) platform to test for HEV viraemia in 2,919 plasma samples collected from HIV-infected men and women enrolled in US cohort studies. Samples were confirmed by real-time polymerase chain reaction. Three samples tested positive for HEV from the cohort of 2,606 HIV+ women, whereas none of the 313 samples collected from HIV+ men had detectable HEV viraemia. All HEV isolates were genotype 3a. Follow-up testing of stored samples revealed that 1 woman had chronic HEV infection for >4 years, whereas 2 women had acute HEV detectable at only a single study visit.

Comment: In addition to its importance as a cause of acute hepatitis, chronic infection with HEV is increasingly recognised as an important cause of morbidity and mortality in the immunosuppressed. This study explored the prevalence of HEV infection in a large cohort of HIV-infected patients in the United States. Only three cases of HEV viraemia, including one case of chronic hepatitis E, were apparent among nearly 3,000 enrollees, suggesting that widespread screening for HEV in HIV-infected persons in the United States is not warranted. The report is of most interest for demonstrating that chronic hepatitis E complicating HIV infection can occur despite a CD4+ T cell count >200 cells/mm3 and in the setting of persistently normal serum AST and ALT values.


Independent commentary by Professor Stephen Riordan.
Senior Staff Specialist, Gastrointestinal and Liver Unit, Prince of Wales Hospital and Conjoint Professor of Medicine, University of New South Wales, Sydney.

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Hepatitis C viral infection increases the risk of lymphoid-neoplasms: a population-based cohort study

Authors: Su TH et al.

Summary: This study investigated the temporal relationship between HCV infection and lymphoid neoplasms using data obtained from the Taiwan National Health Insurance Research Database for the period 2001 through 2005 (HCV cohort). Propensity scores were used to match patients' age, sex, and comorbidities, including rheumatological disorders and diabetes, to another non-HCV cohort. Both cohorts were followed until 2009 for a new diagnosis of any lymphoid neoplasms or non-Hodgkin's lymphoma (NHL). A total of 11,679 HCV and 46,716 non-HCV patients were included and followed for up to 8 years. Incidence rates for any lymphoid neoplasms and NHL were significantly higher in the HCV cohort compared with the non-HCV cohort (48.4 vs 22.1, and 37.0 vs 17.5 per 100,000 person-years, respectively; both p<0.001), even after excluding lymphoid neoplasms developed within the first year of follow-up. In Cox proportional hazards regression analysis, adjusted for age, sex, numbers of annual medical visits during follow-up, and comorbidities, indicated that HCV infection was associated with an increased risk of either any lymphoid neoplasms (HR 2.30; 95% CI, 1.55 to 3.43; p<0.0001) or NHL (HR 2.00; 95% CI, 1.27 to 3.16; p=0.003).

Comment: The possible role of HCV infection in the pathogenesis of lymphoproliferative disorders is controversial. This large Taiwanese nationwide population-based longitudinal cohort study, in which participants were followed for 6 to 8 years, was performed to further investigate the possible association between HCV and lymphoid neoplasms, after adjusting for potential confounding variables. The findings are important in suggesting an approximately two-fold increase in risk of lymphoproliferative disorders, especially NHL, associated with chronic HCV infection. Assessment for lymphoid neoplasms regression with effective anti-HCV therapy, as is now increasingly possible with the availability of direct-acting treatment regimens, will ultimately be required to establish a causal link with HCV.


Perspective on seropositivity among persons vaccinated for hepatitis A during infancy by maternal antibody status: 15-year follow-up

Authors: Spradling PR et al.

Summary: This study examined whether maternal anti-hepatitis A virus (HAV) antibody transfer to children impacts upon the effectiveness of vaccine protection against HAV. A total of 183 Alaskan Native infants and toddlers were randomised by maternal anti-HAV status to initiate a two-dose inactivated hepatitis A vaccine at age 6 months (group 1), 12 months (group 2), or 15 months (group 3). Anti-HAV levels were obtained at intervals through age 15–16 years; seropositivity was less frequent through age 15–16 years; seropositivity (anti-HAV ≥20 mIU/mL) at 30 years after the second vaccine dose among the three groups was predicted using a random effects model. Although the frequency of seropositivity among all participants through age 10 years was high (100% among groups 2 and 3 and >90% among group 1), seropositivity was less frequent through age 15–16 years among those starting vaccination at 6 months (group 1; 50%–75%) and among maternal anti-hepatitis-A-positive children who initiated vaccination at ages 12 months and 15 months (groups 2 and 3, 67%–87%). Nonetheless, the model indicated that anti-HAV seropositivity should persist for ≥30 years after vaccination in 64% of all participants; among those seropositive at age 15–16 years, 84% were predicted to remain so for ≥30 years.

Comment: Among infants born to anti-HAV-positive mothers (due either to natural infection or vaccination, passively acquired maternal anti-HAV antibody may persist until age 12 months and substantially reduce hepatitis A vaccine immunogenicity. This analysis was performed in the light of such concerns. The results are notable in demonstrating that a positive maternal anti-HAV antibody status has a detrimental effect on the duration of a protective antibody response in the offspring following vaccination, even when vaccination occurred at age 12 to 15 months, such that by age 15 to 16 years, one in three such children no longer have protective anti-HAV titres.


Restoration of T cell function in chronic hepatitis B patients upon treatment with interferon based combination therapy

Authors: de Niep A et al.

Summary/Comment: Chronic HBV infection is characterised by a functional impairment of HBV-specific T cells. This study analysed the phenotype and function of HBV-specific CD8+ T cells in chronic HBV-infected patients with a low viral load (as reflected by mean ± SD log10 HBV DNA 2.52 ± 1.15 IU/mL) in comparison to those with a high viral load (as reflected by log10 HBV DNA >4.00 IU/mL) treated with combination pegylated interferon and adefovir. The findings indicate that low viral load patients display HBV core-specific T cells with a memory phenotype and blunted capacity to produce interleukin-2 but strong proliferative potential. Effective antiviral therapy in those with a high baseline viral load leads to a partial recovery of HBV-specific T cells, in which a broader repertoire of HBV-specific T cell functions is restored. The results help explain mechanisms by which antiviral therapy may promote viral clearance in patients with chronic HBV infection.

Reference: J Hepatol. 2016;63(3):539-46

Evidence that hepatitis B virus replication in mouse cells is limited by the lack of a host cell dependency factor

Authors: Lempp FA et al.

Summary: These researchers sought to understand why expression of the specific receptor human sodium taurocholate cotransporting polypeptide (NTCP) in mouse hepatocytes renders them susceptible to hepatitis delta virus (HDV), while HBV remains restricted at an early stage of replication. Analyses were performed upon six NTCP-expressing mouse and human cell lines, which were fused with replication-supporting but non-infectable HepG2 cells. While NTCP expression in three mouse cell lines and the non-hepatitic human HeLa cells conferred susceptibility to HDV, but HBV replication was restricted. All heterokaryotic cells supported HBV replication. NTCP was provided by the mouse cells and replication competence came from the HepG2 cell line. Transfection of non-susceptible cells with a covalently closed circular DNA (cccDNA)-like molecule resulted in the promotion of gene expression, indicating that the limiting step is upstream of cccDNA formation.

Comment: An immunocompetent murine model for HBV infection is currently lacking. Several mouse cell lines reconstituted with NTCP, a receptor located on the surface of hepatocytes that mediates the entry of both HBV and HDV, permit HDV but not HBV infection. This study was performed to investigate this discrepancy. The key finding is that inhibition of HBV infection of not only mouse hepatocyte cell lines but also non-hepatocyte human HeLa cells expressing NTCP is reversed by an intracellular factor(s) expressed by HepG2 cells. The identification of the responsible intracellular factor(s) in future analyses will facilitate the development of a mouse model for HBV infection.

Reference: J Hepatol. 2016;64(3):556-64

Stem cell-derived hepatocytes: A novel model for hepatitis E virus replication

Authors: Helsen N et al.

Summary: This article demonstrates how pluripotent stem cell (PSC)-derived hepatocytes supported the complete HEV replication cycle, infection, replication and generation of infectious virions. Ribavirin and interferon-alpha2b were used to inhibit viral replication. Interestingly, other germ layer cells (PSC-derived mesodermal cells and neuroepithelium) were found to support intracellular replication but were not infectable with HEV. Instead, they only supported HEV replication upon transfection with a HEV subgenomic replicon.

Comment: In vitro culture systems for HEV have been established only recently and are largely based on the use of less than ideal hepatoma cell lines, such that the biology of HEV infection is currently incompletely understood. In this study, the authors demonstrate that human pluripotent stem cell (iPSC)-derived hepatocytes support the complete HEV replication cycle and the generation of infectious virions, rendering this a relevant in vitro model to better understand viral biology and assess potential antiviral treatments. Indeed, the investigators used this model to show that both ribavirin and interferon inhibit HEV replication, extending the results of another recent study that also employed iPSC-derived hepatocytes to assess the effect of ribavirin and sofosbuvir on HEV replication.

Reference: J Hepatol. 2016;64(3):565-73

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97% \((n=1062/1096)\) achieved SVR12

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HCV=Hepatitis C Virus. RNA=Ribonucleic Acid. SVR=Sustained Virologic Response. GT1=Genotype 1. RBV=Ribavirin.
Hepatitis B virus-specific and global T-cell dysfunction in chronic hepatitis B

Authors: Park J-J et al.

Summary: This study involved 200 adults with chronic HBV who participated in the National Institutes of Health-supported Hepatitis B Research Network from 2011 through 2013 and 20 uninfected individuals (controls). Peripheral blood lymphocytes were analysed from all participants for T-cell responses (proliferation and production of interferon-γ and interleukin 10) to overlapping HBV peptides (preS, S, preC, core, and reverse transcriptase), influenza matrix peptides, and lipopolysaccharide. Flow cytometry analysed T cell expression of regulatory markers FOXP3, programmed death-1, and cytotoxic T lymphocyte-associated antigen-4. Moreover, the HBV core-specific T cell response was weaker in HBBeAg+ patients compared with HBBeAg- patients (percent responders: 3% vs 23%, p=0.000008). While in vitro blockade of programmed death-1 or cytotoxic T lymphocyte-associated antigen-4 increased T cell responses to HBV, the effect was smaller in HBBeAg+ compared with HBBeAg- patients. Furthermore, T cell responses to influenza and lipopolysaccharide were weaker in the chronic HBV cohort compared with controls.

Comment: This important study was conducted based upon a hypothesis that the various clinical phases of chronic hepatitis B can be defined by immune effector and regulatory factors that impact upon HBV-specific T cell function. This proved to be not the case, since T cell-related effector and regulatory mediators neither correlated significantly with clinical parameters nor provided distinct immune signatures for the various clinical phases of chronic HBV infection. Multiple immune-regulatory pathways were found to be induced by chronic HBV infection, leading to a reversible suppression of not only HBV-specific T cell but also global effector T cell function. This is the first clinical study to suggest that HBBeAg contributes to this suppression of T cell function in chronic HBV infection, with, in particular, proliferative, interferon-γ and interleukin-10 responses to HBV core antigen all markedly reduced in HBBeAg+ patients compared with HBBeAg- counterparts.

Reference: Gastroenterology. 2016;150(3):684-95

Abstract

Virus-specific CD4+ T cells have functional and phenotypic characteristics of follicular T-helper cells in patients with acute and chronic HCV infections

Authors: Raziorrouh B et al.

Summary: Blood samples from patients with acute and chronic HCV infection and healthy individuals (controls) underwent MHC class II tetramer analysis, assays to detect intracellular cytokines in response to HCV exposure, and analyses to quantify HCV-specific antibodies. Liver tissues were obtained from patients with chronic HCV infection or non-viral liver disease, in order to analyse markers of follicular T helper (Th9) cells.

Comment: Data derived from murine models suggest that Th9 cells are important to contain viral infections. Patients with acute HCV infection who develop vigorous and multi-specific CD4 T cell responses are more likely to spontaneously clear the infection. This study was performed to analyse Th9 signatures on virus-specific CD4+ T cells of patients with acute and chronic HCV infection. Virus-specific peripheral blood CD4+ T cells expressed Th9 markers in patients with acute HCV infection, but these markers were barely detectable in patients with chronic HCV infection. High levels of interleukin-21 secretion by CD4+ T cells during acute HCV infection point to an active role for this Th9 signature cytokine during acute, rather than chronic, HCV exposure, although further studies will be required to better understand the full mechanistic relevance of Th9 cells to HCV clearance.


Abstract

Serum viral duplex-linear DNA proportion increases with the progression of liver disease in patients infected with HBV

Authors: Zhao XL et al.

Summary: For this investigation, the researchers developed a peptide nucleic acid-mediated quantitative real-time PCR clamping assay to measure the proportions of duplex-linear DNA (dlDNA) in total HBV DNA in sera obtained from patients with chronic HBV infection, liver cirrhosis or hepatocellular carcinoma (HCC). The study also sought to determine what factors influence the proportion of dlDNA relative to total HBV DNA. In patients with chronic HBV infection, the average dlDNA proportion was approximately 7% and was elevated in those with abnormal levels of alanine aminotransferase. Sera dlDNA proportions increased to approximately 14% and 20% in patients with liver cirrhosis and HCC, respectively. Interferon-α treatment slightly increased the dlDNA proportion in the responders; nucleotide analogue therapy spuriously elevated the proportion. The dlDNA proportion was significantly altered when human hepatoma cells supporting HBV replication were exposed to inflammatory cytokines.

Comment: Viral dlDNA, accounting for only a minor proportion of total HBV DNA, more readily integrates into chromosomes than predominating relaxed-circular DNA. This study used a novel molecular assay to investigate the proportion of HBV dlDNA relative to HBV total DNA in patients with chronic HBV infection and to determine, for the first time, that this proportion increases with both progression of liver disease and development of hepatoma. The findings further suggest that chronic hepatic inflammation may elevate the dlDNA proportion, raising the possibility of a novel interaction between HBV and immunity by which the immune response induced by HBV may alter the composition of the HBV genome.


Abstract

Chimeric antigen receptor (CAR)-engineered T cells redirected against hepatitis C virus (HCV) E2 glycoprotein

Authors: Sautto GA et al.

Summary: These Italian investigators acknowledge the significant advances in the treatment, recovery and life expectancy of patients with HCV infection, due to novel therapeutic approaches such as direct-acting antivirals. However, alternative strategies are needed for non-responding or relapsing patients. This article describes promising antiviral activity of chimeric antigen receptor (CAR)-grafted T cells targeting the HCV E2 glycoprotein (HCV/E2). Anti-HCV/E2 CARs were composed of single-chain variable fragments obtained from a broadly cross-reactive and cross-neutralising human monoclonal antibody, e137, fused to the intracellular signalling motif of the co-stimulatory CD28 molecule and the CD3ζ domain. Activity of CAR-grafted T cells was evaluated in vitro against HCV/E2-transfected cells as well as hepatocytes infected with cell-culture-derived HCV.

Comment: The use of CARs to arm T cells against highly conserved HCV antigens may represent a novel approach to antiviral therapy. This proof-of-concept study describes for the first time the retroviral engineering of healthy donor T cells with CARs targeting a highly conserved epitope of HCV/E2 glycoprotein, a major target of the host immune response, in order to harness the cellular and humoral arms of the immune response in a single approach. Such anti-HCV CAR-modified T cells were found to be capable of secreting pro-inflammatory and antiviral cytokines and lysing HCV/E2-expressing cells and HCV-infected hepatocytes. Although these preliminary results appear promising, the safety of bioengineered T cells will have to be carefully assessed before any possible clinical application.


Abstract