Editorial

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Paving the way for T cell-based immunotherapies in chronic hepatitis E

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Hepatitis E virus (HEV) causes an estimated 3 million cases of acute symptomatic hepatitis annually, which may have a severe course, leading to approximately 70,000 HEV-related deaths per year.[1] Pregnant women are at the highest risk of a fulminant course upon infection with HEV genotypes 1 and 2, which are spread as waterborne pathogens in low-income regions. In middle- and high-income regions, severe courses of acute hepatitis E are usually observed in elderly people, following infection with the zoonotic HEV genotypes 3 and 4 that are transmitted by meat and less frequently by blood products. Next to acute hepatitis, HEV can also cause severe extrahepatic manifestations, including Guillain-Barré syndrome.[2] In addition, HEV genotypes 3 and 4 can cause persistent infection in immunosuppressed hosts, usually following solid organ or stem cell transplantation. These patients may clear HEV infection after reduction of their immunosuppressive regimen or after antiviral treatment with ribavirin. Those immunosuppressed patients who fail to eliminate HEV, however, are at a high risk of developing cirrhosis and liver failure within a relatively short time span.[1] Thus, novel antiviral strategies are urgently needed for these patients. Indeed, immunotherapy may be an attractive approach in this context.

HEV-specific antibodies have been shown to mediate neutralizing immunity in most patients after resolved infection and also upon vaccination by the recombinant HEV vaccine that has been approved in China.[3] However, antibodies have not been shown to have a role in viral clearance following acute infection, or upon reduction of immunosuppression or administration of ribavirin in chronic infection. Indeed, virus-specific T cells are generally thought to play the dominant role in viral clearance in acute viral hepatitis. This assumption is in line with the observation that HEV *per se* is non-cytopathic in cell culture,[4,5] pointing at immune-mediated hepatic injury, as well as the observation that HEV replication in cell culture is sensitive to T cell-produced cytokines such as IFN-γ.[6] Virus-specific T cells may also play a driving role in immunopathogenesis of both, acute and chronic hepatitis, including extrahepatic manifestations. Despite this supposed preeminent role in the natural history of acute and chronic HEV infection, there is a remarkable paucity of studies addressing HEV-specific T cell responses.[3] Indeed, early studies using recombinant HEV capsid protein (open reading frame [ORF]2)[7–9] as well as more recent studies using overlapping peptides covering some (ORF2 and ORF3)[10,11] or all HEV domains (ORF1, ORF2, and ORF3)[12,13] showed broad and vigorous HEV-specific CD4+ and CD8+ T cell responses in immunocompetent patients with acute hepatitis E that declined over time after viral clearance. In contrast, immunosuppressed patients with chronic HEV infection displayed a narrow and weak HEV-specific T cell response that was at or below the limit of detection in most patients. Checkpoint inhibitors targeting PD-1 or CTLA-4, as well as successful antiviral treatment, at least partially restored T cell responses in some of these patients.[11,14] Until now, however, not a single HEV-specific CD4+ or CD8+ T cell epitope had been defined in detail, including optimal peptide length as well as HLA class I or II restriction. In consequence, no studies using HLA class I or II multimers allowing direct *ex vivo* detection and multiparameter analyses of HEV-specific CD8+ and CD4+ T cells on a single-cell level could be performed. Thus, there is a remarkable deficit in knowledge about the contribution of HEV-specific T cells to viral clearance, pathogenesis in acute infection, especially in the severe clinical courses observed in pregnant women and elderly persons, liver damage in chronically infected immunosuppressed individuals, and extrahepatic manifestations.[3] In addition, elemental requirements for the development of immunotherapeutic approaches in chronic HEV infection could not be addressed. For example, since no HEV-specific HLA class I multimers have been available, HEV-specific CD8+ T cells could not be isolated to allow analysis of their T cell receptor (TCR) repertoire and subsequent redirection of non-specific, but functional CD8+ T cells. Similarly, the exact mechanisms of HEV-specific T cell exhaustion/deletion in chronic HEV infection could not be studied and thus promising methods for HEV-specific T cell restoration (*e.g.* anti-PD1/PD-L1, anti-CTLA-4, pro-inflammatory cytokines etc.) have not been studied in detail.

In this issue of the *Journal of Hepatology*, Soon *et al.* analyzed HEV-specific CD8+ T cell responses in 12 HLA-A*02+ patients

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with acute HEV genotype 3 infection.[15] Similar to previous studies, the authors used overlapping peptides spanning all HEV domains (ORF1, ORF2, and ORF3). In contrast to previous reports, however, the authors went further and defined optimal 9-mer epitopes restricted by HLA-A*02 that corresponded to the positive overlapping peptides. Indeed, the authors predicted 12 HLA-A*02-restricted HEV-specific CD8+ T cell epitopes, 8 located in ORF1 and 4 located in ORF2. Three of these optimal epitopes could be confirmed by HLA-A*02:01 multimer staining in patients with acute HEV infection: ORF1-1116 (SLFWNEPAI) located in the RNA helicase domain of ORF1, ORF1-1527 (LLWNTVWNM) located in the RNA-dependent RNA polymerase domain of ORF1, and ORF2-493 (YVSDTVTFV) located in the capsid protein (ORF2). Responses against these HEV-specific CD8+ T cell epitopes were vigorous in some of the 12 HLA-A*02+ patients with acute-resolving HEV infection, however, they could hardly be detected in any of 9 HLA-A*02+ immunosuppressed patients with chronic HEV infection. Checkpoint inhibition by anti-PD-L1 did not result in a substantial restoration of these responses. In the next step, the authors sorted epitope-specific CD8+ T cells from 4 patients with acute or resolved HEV infection and performed next generation sequencing of the TCR repertoire. Interestingly, the TCR α-chain variable gene (TCRAV) 12-02 was present in all 4 patients at frequencies ranging from 12–80%, indicating that it may have an important role in HEV control. The authors then redirected non-specific CD8+ T cells from both, HLA-A*02+ healthy controls as well as HLA-A*02+ patients with chronic HEV infection using synthetic mRNA coding for the HEV-specific TCR genes sequenced from the patients with acute HEV infection. Electroporation was used for transient TCR redirection. These redirected, HEV-specific CD8+ T cells generated *in vitro* displayed a high epitope-specificity in HLA-A*02 tetramer staining, a polyfunctional cytokine profile upon peptide stimulation, and sensitive and specific killing of peptide-loaded target cells. Importantly, a similar quality of HEV-specific CD8+ T cells could be generated from chronically infected immunosuppressed patients that were previously refractory to PD-L1 blockade. These results indicate that treatment of chronic HEV infection with *in vitro* redirected HEV-specific T cells may be feasible. A similar approach has been successfully tested for hepatitis B virus (HBV) clearance in mice.[16] In a pioneer human study, HBV-specific redirected CD8+ T cells have been used in a single patient with HBV-induced end-stage hepatocellular carcinoma.[17] While this approach led to a convincing immunological response, the patient died soon after because of end-stage cancer.

This study raises some specific as well as general questions concerning the method of TCR redirection that should be considered and addressed in the future. First, the study was not designed to define the complete breadth and dominance pattern of HEV-specific CD8+ T cell epitopes. Indeed, the study was limited to HLA-A*02-restricted CD8+ T cell epitopes, and the epitopes confirmed here were not targeted in the majority of patients with acute HEV infection. Thus, future studies need to identify immunodominant HEV-specific CD8+ T cell epitopes. These may have a dominant role in viral control and may be restricted by HLA class I alleles other than HLA-A*02. Of note, the impact of viral escape in chronic HEV infection also needs to be defined, since redirection of T cells against a mutated viral epitope may be of no help. In addition, a wisely directed regimen of checkpoint inhibition and additional methods to restore virus-specific T cells, such as pro-inflammatory cytokines, may be needed for ultimate success.[18] The use of TCR-redirected CD8+ T cells with the risk of uncontrolled proliferation and overwhelming cytotoxicity in humans also raises safety concerns. Importantly, the authors generated redirected CD8+ T cells that only transiently express virus-specific TCRs by using mRNA electroporation. This method of T cell redirection may limit safety concerns when compared to permanent T cell redirection by transduction with viral vectors.[19] In addition, the selection of CD8+ T cells with dominantly non-cytolytic effector functions may further reduce risks.[20] These important issues regarding epitope selection as well as safety concerns should be taken into account before the work- and cost-intensive procedure of TCR redirection may be translated to chronic HEV infection in the clinic. In the authors' own words, however, this study has "set in motion the initial groundwork for a new approach towards T cell-based therapies in chronic HEV infection". Hopefully these therapies will provide a cure for patients with chronic HEV infection who have experienced treatment failure with the current antiviral treatment options.

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Conflict of interest

The author declares no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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