nature reviews gastroenterology & hepatology

https://doi.org/10.1038/s41575-024-00950-z

Review article

Check for updates

Hepatitis E virus: from innate sensing to adaptive immune responses

Yannick Brüggemann [1,6], Mara Klöhn [1,6], Heiner Wedemeyer [2,3,4] & Eike Steinmann [1,5]

Abstract

Hepatitis E virus (HEV) infections are a major cause of acute viral hepatitis in humans worldwide. In immunocompetent individuals, the majority of HEV infections remain asymptomatic and lead to spontaneous clearance of the virus, and only a minority of individuals with infection (5–16%) experience symptoms of acute viral hepatitis. However, HEV infections can cause up to 30% mortality in pregnant women, become chronic in immunocompromised patients and cause extrahepatic manifestations. A growing body of evidence suggests that the host immune response to infection with different HEV genotypes is a critical determinant of distinct HEV infection outcomes. In this Review, we summarize key components of the innate and adaptive immune responses to HEV, including the underlying immunological mechanisms of HEV associated with acute and chronic liver failure and interactions between T cell and B cell responses. In addition, we discuss the current status of vaccines against HEV and raise outstanding questions regarding the immune responses induced by HEV and treatment of the disease, highlighting areas for future investigation.

Sections

[1]Department of Molecular and Medical Virology, Ruhr University Bochum, Bochum, Germany. [2]Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany. [3]German Center for Infection Research (DZIF), Partner Sites Hannover-Braunschweig, Hannover, Germany. [4]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany. [5]German Center for Infection Research (DZIF), External Partner Site, Bochum, Germany. [6]These authors contributed equally: Yannick Brüggemann, Mara Klöhn. e-mail: eike.steinmann@ruhr-uni-bochum.de

Key points

- The severity of hepatitis E virus (HEV) infection varies: for example, it is self-limiting in most immunocompetent individuals but can be fatal in pregnant women and lead to chronicity in immunocompromised individuals.
- A growing body of evidence suggests that the host immune response to infection with different HEV genotypes is a critical determinant of the distinct outcomes of HEV infection.
- HEV has developed strategies to evade and disrupt innate immune signalling by antagonizing interferon induction and signalling.
- T cell immunity is of paramount importance in the resolution of HEV infections.
- B cell immunity and antibody responses can provide sterilizing immunity and long-lasting protection against HEV re-infection.
- Active and passive immunization seem to effectively prevent severe acute hepatitis E in most cases.

Introduction

Hepatitis E virus (HEV) is an understudied RNA virus and is currently the major causative agent of acute viral hepatitis in humans worldwide. At least 20 million HEV infections occur annually, accounting for approximately 3.3 million cases of acute illness and 44,000–70,000 deaths[1–3]. The virus was initially described during a waterborne epidemic of acute non-A, non-B hepatitis in the 1980s[4]. Although tremendous advancements have been made over the past few decades (Fig. 1), many aspects of HEV infection biology and pathogenesis remain poorly understood.

HEV is a single-stranded positive-sense RNA virus belonging to the *Hepeviridae* family[5]. The genome of HEV encompasses three open reading frames (ORFs) that encode non-structural proteins (ORF1), comprising a methyltransferase, a putative papain-like cysteine protease (PCP), helicase and RNA-dependent RNA polymerase; a capsid protein (ORF2); and a small membrane-associated protein required for infectious particle secretion (ORF3)[6,7]. The majority of HEV infections in humans are caused by four different genotypes: HEV1–HEV4 (ref. 5). HEV1 and HEV2 are obligate human pathogens that are endemic to low-income countries and are mainly transmitted by the faecal–oral route by contaminated drinking water. Clinically evident hepatitis arises from approximately 16% of acute HEV1 or HEV2 infections[8], which can lead to severe hepatitis and mortality during pregnancy[9]. In contrast, HEV3 and HEV4 are zoonotic pathogens with broad host ranges, including swine, deer and rabbits, which serve as reservoirs for HEV in high-income countries, causing sporadic cases of enterically transmitted, zoonotic hepatitis E[6,10]. Acute HEV3 or HEV4 infection remains clinically asymptomatic in the vast majority of patients, with only a minority of patients (HEV3 <5%; HEV4 <3%) developing symptoms of acute hepatitis E, with elevated liver enzymes, jaundice and non-specific symptoms such as fatigue, itching and nausea lasting days to several weeks[11–13] (Fig. 2a). Incubation periods for HEV typically range between 4 and 6 weeks from infection to the onset of symptoms[14,15] (Fig. 2a). Given that HEV replication and infection are non-cytopathic[16], and the onset of icteric symptoms typically occurs in conjunction with an increase in antibodies and a decrease in viral load (Fig. 2a), the immune response seems to be an important determinant driving hepatitis E pathogenesis[17].

Distinguishing acute hepatitis E from other types of viral hepatitis is challenging as there are no distinctive clinical symptoms specific for HEV[8]. Before chronic hepatitis E cases were reported among immunocompromised individuals[18,19], HEV was thought to resemble hepatitis A virus (HAV) infections — acute, self-limiting infections that are usually transmitted by the faecal–oral route[20,21]. Similar to HAV, HEV is released non-lytically from hepatocytes as 'quasi-enveloped' virions cloaked in host membranes[22]. While possessing infectious properties, these membrane-encased virions differ from typical enveloped viruses, such as coronaviruses or hepatitis B virus (HBV), as they lack virally encoded proteins on their surface and are thereby resistant to neutralizing anti-capsid antibodies induced by infection[22]. While enveloped viruses circulate in the blood and spread within the liver, their membranes are lost during transit through the digestive system and shed as naked viruses. Unlike HAV, chronic HEV3 or HEV4 infections, which potentially result in cirrhosis, have been reported in immunocompromised patients, including organ transplant recipients[18], patients receiving cancer chemotherapy[23] and people living with HIV[24] (Fig. 2a). Moreover, in contrast to HAV and all other major hepatitis viruses (HBV, HCV and HDV), HEV can infect various animal species, and zoonotic transmission to humans is not uncommon[6,10].

In addition to hepatitis, HEV infection has been linked to several extrahepatic manifestations, including neurological diseases (reviewed previously[25]), renal disease and acute pancreatitis (reviewed previously[26]). Current therapeutic options against HEV are limited to the off-label use of the broad-spectrum antiviral agent ribavirin and, in some instances, pegylated IFNα[12,27]. However, interferon can increase the risk of acute rejection in transplant recipients, while ribavirin therapy is contraindicated in pregnancy owing to its teratogenicity[12]. In addition, suboptimal efficacy, poor tolerability and adverse effects further limit the use of both treatment options. Furthermore, HEV variants in response to antiviral treatment have been identified[28–30]. Although the emergence of viral variants could potentially have a role in ribavirin resistance, a causal link to treatment failure has yet to be firmly established.

The exact reasons for the varying degrees of disease severity and the pathogenesis of HEV infections remain unclear. During infection, a range of cellular receptors and/or sensors can detect the presence of HEV, initiating an interferon and inflammatory response to counteract HEV. However, dysregulation of immune responses (that is, abnormal secretion of pro-inflammatory cytokines and chemokines) during infection might contribute to pathogenic effects such as tissue damage and liver inflammation. In agreement with this, a growing body of evidence implies that the host immune response to infection with different HEV genotypes is a critical determinant of the distinct outcomes of HEV infection[17,31]. Hence, understanding how HEV and its different genotypes regulate innate and adaptive immune responses is essential to guiding improvements in HEV therapy, developing effective vaccine applications, and designing new therapeutic strategies to control infection and suppress liver disease. In this Review, we summarize key features of the innate and adaptive immune responses to HEV infection. We further discuss how these virus–host interactions could affect the outcome of infection and immunity as well as the current status of vaccines against HEV.

Innate immunity to HEV

Detection of HEV by pattern recognition receptors

As the first line of host defence, the innate immune system responds rapidly but non-specifically to viral infections[32]. Following infection, HEV is

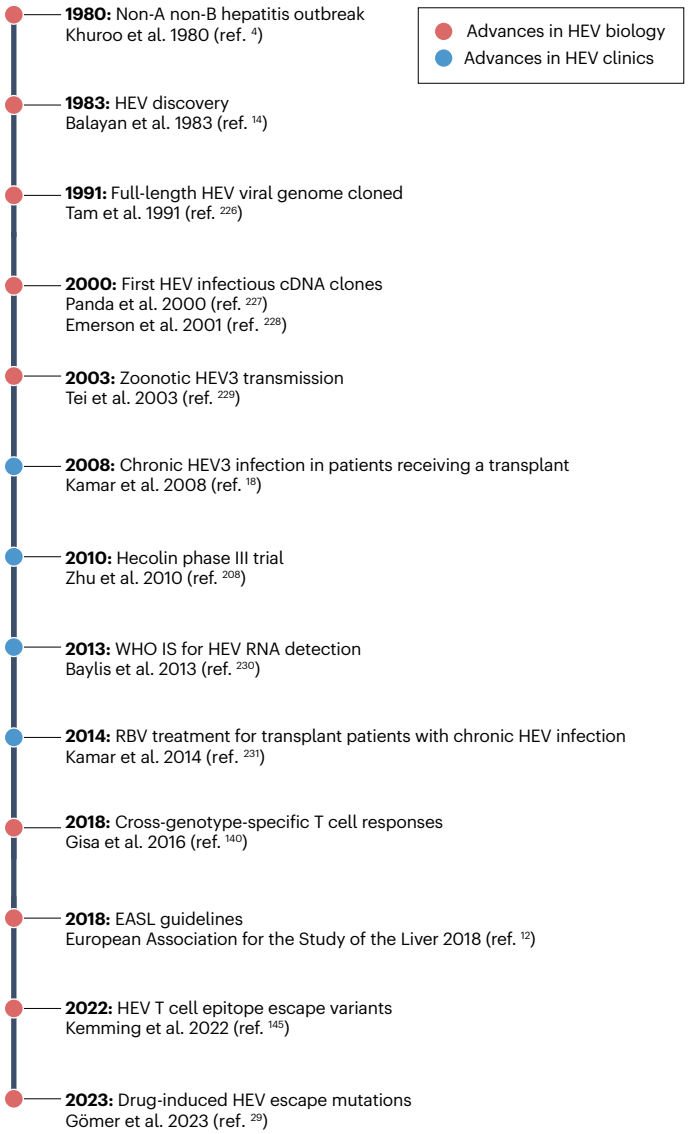


Fig. 1 | Timeline of milestones in basic and clinical HEV research. Hepatitis E virus (HEV) came to light during an epidemic of non-A, non-B hepatitis in Kashmir, India, in 1978 (ref. 4), and in 1983 the viral agent responsible was discovered[14]. Molecular cloning of the HEV genome followed a few years later[225,226], eventually leading to the development of the first infectious molecular clones of HEV[227,228]. These findings were followed by the recognition of zoonotic transmission[229] and the first observations of chronic infections in immunocompromised transplant recipients[18]. Clinical advances in HEV research include the successful development of a recombinant hepatitis E vaccine[208], implementation of the WHO International Standard (IS) to harmonize assays for the detection of HEV RNA[230], the first successful treatment of chronic HEV infections with ribavirin (RBV) monotherapy[231], and demonstration of cross-genotype-specific T cell responses[140], culminating in the clinical practice guidelines for the treatment and management of hepatitis E[12]. However, the identification of treatment-associated[28–30] and T cell immune escape variants[145] highlights the need to develop novel HEV therapies and the need to understand the underlying components of the immune system required for virus clearance. EASL, European Association for the Study of the Liver.

sensed as non-self by pattern recognition receptors (PRRs) in the host cell, which bind to pathogen-associated molecular patterns (PAMPs) associated with viral replication, leading to activation of the innate immune response[16,33] and promoting priming of adaptive immune responses[34]. Hepatocytes, the primary cell type infected by HEV, express both cytosolic retinoic acid-inducible gene I (RIG-I)-like receptors and membrane-bound Toll-like receptors (TLRs), which reside on the cell surface as well as in endosomes[35]. TLR signalling upon HEV recognition is initiated through the recruitment of specific adaptor molecules, such as myeloid differentiation primary response 88 (MYD88)[32,36], and results in activation of nuclear factor κB (NF-κB)[37], activator protein 1 (AP1) and interferon regulatory factors (IRFs)[36], which induce inflammatory cytokine expression[38]. The engagement of TLRs has been supported by in vitro data, indicating a role for TLR2, TLR4 and TLR3 in sensing HEV replication intermediates and capsids to elicit an inflammatory response[36,39]. Accordingly, peripheral blood mononuclear cells (PBMCs) in patients with acute HEV infection expressed higher levels of TLR3 and pro-inflammatory and anti-inflammatory cytokines (IFNγ, TNF, IL-10 and TGFβ) compared with patients with acute liver failure[40], implying a delicate cytokine balance to counter viral infection while also restricting excessive inflammation. Additionally, monocytes and macrophages in pregnant women with acute liver failure showed reduced TLR3 and TLR7 expression, suggesting a crucial role for TLRs in curbing viral infection[41]. Susceptibility to HEV infection in a cohort of Indian patients has been linked to a TLR4 polymorphism (T399I) associated with receptor hypo-responsiveness[42]. Collectively, these data support an important role for TLRs in recognizing HEV-associated molecular patterns and initiating an innate immune response. In addition to TLR sensing, in vitro studies have implicated the RIG-I-like receptor signalling mediators RIG-I, melanoma differentiation-associated protein 5 (MDA5) and mitochondrial antiviral-signalling protein (MAVS) in sensing HEV RNA and triggering an antiviral interferon response[16,43–45]. Knockdown of RIG-I, MDA5 and MAVS in hepatic liver cell lines revealed a reduction of HEV-induced type III interferons[46], while overexpression of either MDA5 or RIG-I resulted in reduced HEV replication[43,44]. Moreover, increased RIG-I expression has been observed in liver tissues of rhesus macaques infected with either HEV1 or HEV3 (ref. 37). RIG-I primarily detects short double-stranded RNA with 5′ triphosphate groups, whereas MDA5 primarily recognizes long double-stranded RNA[47]. Upon RNA binding, both RIG-I and MDA5 undergo conformational changes and recruit MAVS to assemble a signalling complex, which activates IRF3 and IRF7 as well as NF-κB, leading to interferon expression and release. Treatment of HuH-7 S10-3 liver cells with various HEV RNA PAMPs suggests that the U-rich region in the 3′ UTR of the HEV genome functions as a potent RIG-I PAMP[48]. As the length of poly(A) tails can vary among HEV genotypes, and longer poly(A) tails might enhance interferon induction, as observed for HCV[49,50], variability in interferon induction might depend on the viral RNA sequence[48]. On the other hand, single-stranded HEV RNA has been observed to trigger an antiviral response independently of viral replication[45]. However, the exact receptors or sensors that recognize HEV PAMPs, and whether the generation of replicative intermediates is required to induce an innate immune response, remain unclear. PRR sensing of HEV converges in the activation of downstream signalling cascades, including the IκB kinase (IKK) complex and TANK-binding kinase 1 (TBK1) as well as transcription factors such as IRF3 or IRF7 (ref. 45) and NF-κB[36,51], resulting in cytokine release and inflammasome activation in vitro (Fig. 2b).

Interferon response to HEV infection

HEV-induced activation of host innate immunity has been widely observed in various in vivo and in vitro models[16,52,53]. Accordingly, phosphorylation of signal transducer and activator of transcription 1 (STAT1), a hallmark of activation of the antiviral interferon response, has been detected in liver tissues of patients with HEV infection[45], as has elevated interferon-stimulated gene (ISG) expression in the sera of patients with chronic HEV infection[54]. Within hepatocytes and enterocytes, HEV predominantly induces a type III interferon response[46,55,56], and, accordingly, high serum IFNλ3 levels have been observed in patients with acute HEV infection[57]. Similarly, liver tissues of pigs infected with HEV3 showed a type III interferon response, while a type I interferon response was noted in HEV-infected pig enterocytes[48]. Interestingly, gene expression profiles of livers from experimentally infected rhesus macaques showed substantial differences in the timing and magnitude of expression of host immune response-related genes upon infection with patient-derived HEV1 and HEV3 isolates, including downregulation of immune response-related genes for HEV1 (ref. 37). HEV1 showed higher levels of replication and release of several pro-inflammatory cytokines in primary human placental tissue explants compared with HEV3, while HEV1 viral load was negatively correlated with the expression of type III interferons[58]. Likewise, HEV1 displayed greater infectivity and virulence in non-human primates[59] and replicated more efficiently in mice with chimaeric human livers compared with HEV3 (refs. 52,60,61). Elevated levels of viral replication and cytokine release (IL-6, CCL3 and CCL4), particularly in placental tissue explants[58], could potentially offer an explanation for the differing outcomes observed between HEV1 and HEV3 infections during pregnancy. Whether the lower replication efficiency of HEV3 stems from a more robust host innate response or an inherent characteristic of the virus, and how this relates to the capacity of HEV3 to cause chronic infections in immunocompromised individuals, remain unknown. Given that interferon production during infection seems to be dependent on genotype and tissue[48,58,62], it will be important to further study how genotype-specific replication capacities and dysregulation of the local secretome relate to distinct clinical outcomes in the future.

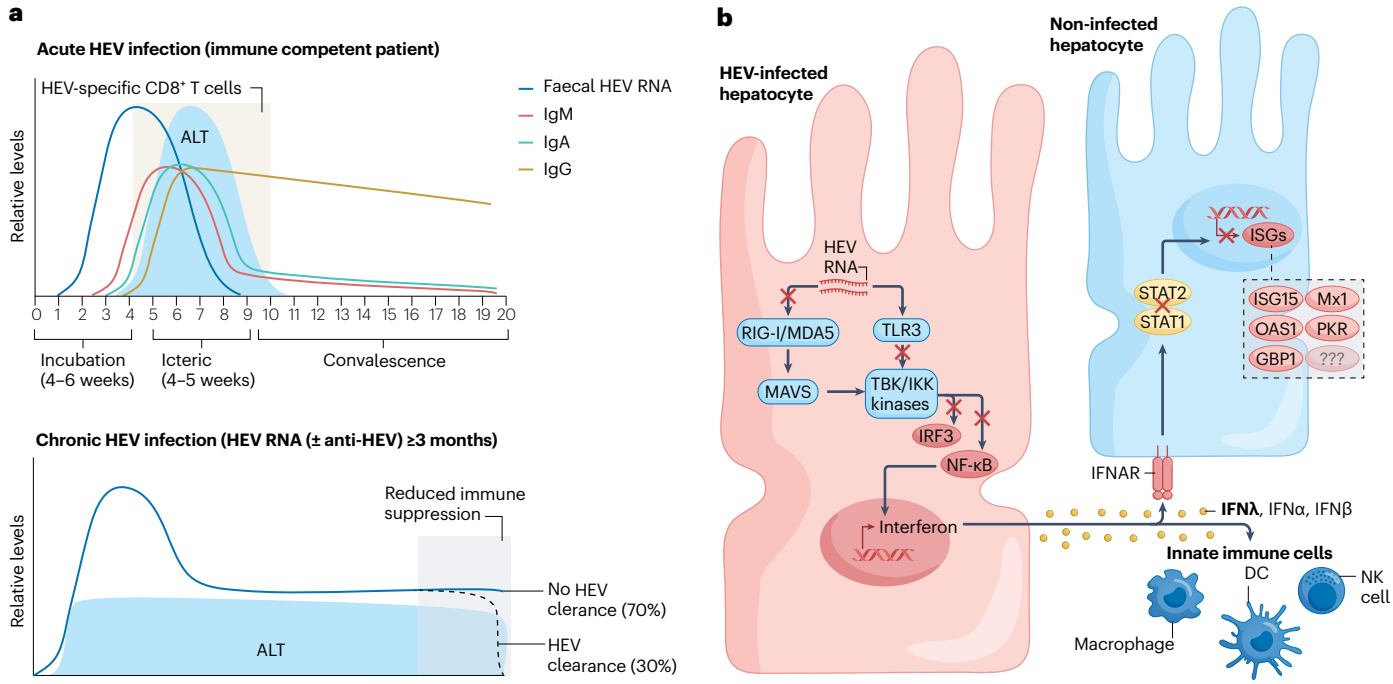


Fig. 2 | HEV disease course and innate immune response in hepatocytes. **a**, Virological and serological markers in acute (top) and chronic (bottom) hepatitis E. Acute hepatitis E virus (HEV) infection involves an incubation phase of 4–6 weeks, which in rare cases is followed by an icteric period (4–5 weeks)[15]. The anti-HEV antibody response involves an early increase in IgA and IgM, which transits into a durable IgG response following clearance and convalescence. HEV-specific CD8⁺ T cell activity has been observed in patients with acute infection[135,136]. Chronic HEV infections have been observed in individuals with compromised immunity[18]. A reduction of immunosuppressive medication results in HEV clearance in approximately one-third of patients[15]. Humoral and cellular responses during chronic infection are highly variable[15], and different timings and magnitudes of seroconversion (if any) have been observed. **b**, Interplay between innate immunity and HEV. HEV RNA and replication intermediates are sensed within the cytoplasm by RIG-I and MDA5, as are endosomes by Toll-like receptor 3 (TLR3). Subsequent activation of kinases, such as the IKK complex and TBK1, promotes the activation of transcription factors, such as nuclear factor-κB (NF-κB) and IRF3, resulting in type I and type III interferon production. Upon release, interferons promote the activation and recruitment of innate immune cells and induce the expression of proviral and antiviral interferon-stimulated genes (ISGs) via JAK–signal transducer and activator of transcription (STAT) signalling in both an autocrine and paracrine manner[33]. HEV interferes with various steps of the cellular innate immune response (red stop signs), including with pattern recognition receptor activation and signalling, IRF3 and NF-κB transcriptional activity, as well as interferon-induced STAT phosphorylation and subsequent ISG expression[85,102,107]. ALT, alanine aminotransferase; DC, dendritic cell; GBP1, guanylate-binding protein 1; IFNAR, type I interferon receptor; IKK, IκB kinase; IRF, interferon regulatory factor; MAVS, mitochondrial antiviral-signalling protein; MDA5, melanoma differentiation-associated protein 5; NK, natural killer; OAS1, oligoadenylate synthetase-related domain 1; PKR, protein kinase R; RIG-I, retinoic acid-inducible gene I; TBK1, TANK-binding kinase 1.

ISG expression during HEV infection

Upon release, interferons trigger the transcription of ISGs, which initiate antiviral effector functions in infected and uninfected neighbouring cells as well as mobilizing adaptive immune responses. Transcriptional profiles from kidney transplant recipients with chronic HEV infection showed expression of 25 genes annotated as ISGs, including those encoding interferon-induced proteins with tetratricopeptide repeats (IFIT1, IFIT2, IFIT3 and IFIT5), 2′-5′-oligoadenylate synthetase-related domains (OAS2, OAS3 and OASL) as well as the ubiquitin-like modifier ISG15 and Mx1 (ref. 63). Many viruses have a unique antiviral 'ISG profile', and selected ISGs can also facilitate replication of a given virus[64,65]. Although the HEV-induced expression of ISGs has been described in various in vitro[16,36,46,58,66] and in vivo models[37,53,67], specific anti-HEV properties have only been confirmed for a small number of these ISGs (Fig. 2b). The PRR RIG-I was found to exert a strong antiviral activity against HEV in human and mouse cell lines by triggering the transcription of a wide range of genes, including ISGs, and interferon production[43]. Likewise, overexpression of IRF1 in different human cell lines (HuH-7, HepaRG, A549, MRC) was found to induce the expression and phosphorylation of STAT1, resulting in the induction of downstream ISGs and lowered HEV replication[68]. The interferon-induced guanylate-binding protein 1 (GBP1) has been shown to potently restrict HEV infection by potentially altering the subcellular localization of the HEV capsid protein towards lysosomes[69]. On the other hand, ISG15 has been suggested to have an immunomodulatory role during HEV infection as loss of ISG15 had no major effect on HEV replication but enhanced the type I interferon-mediated antiviral response by upregulating Mx1, OAS1 and protein kinase R (PKR) expression in hepatic liver cell lines[67,70]. Interestingly, ISG15 deficiency in humans has been linked to persistent interferon expression and increased antiviral response to different viruses[71]. Nonetheless, despite these notions, detailed molecular mechanisms for the different ISGs remain sparse. Thus, identifying the specific ISGs that modulate HEV replication, evaluating their mechanisms of action, and exploring their potential role as tissue-specific restriction factors will be an important next step towards a deeper understanding of HEV–host interactions and potentially novel avenues for antiviral drug development[72].

Inflammatory response upon HEV infection

Inflammatory responses coordinated by the secretion of cytokines and chemokines prevent the spread of pathogens but can cause tissue damage and/or pathogenesis upon dysregulation[73,74]. Histological evidence of liver inflammation has been observed in HEV3-infected or HEV4-infected rabbits and hospitalized patients with HEV infection[75]. Similarly, increased levels of inflammatory cytokines, including IFNγ, TNF, IL-10 and IL-18, have been associated with acute HEV3 infection[76], HEV3-associated liver failure[77] and adverse pregnancy outcomes in patients[78]. Pharmacological inhibition of TNF in a patient with psoriatic arthritis or JAK inhibition in a patient with autoimmune inflammatory rheumatic disease have been linked to increased HEV susceptibility and/or exacerbation of infection and facilitation of HEV infection in vitro[79,80]. Polymorphisms in the promotor regions of TNF have been associated with higher susceptibility (TNF-308-AA) or clinically more apparent HEV infections (TNF-1031-CC)[81] as well as adverse pregnancy outcomes[82]. Overall, these observations highlight the intricate balance between innate immune signalling events that control HEV infection and dysregulated cytokine release, resulting in HEV-induced pathogenesis.

HEV evasion from innate immune signalling

The absence of a robust type I interferon response in HEV-infected human cells[46,55,56], combined with relatively low levels of ISG induction in HEV-infected chimpanzees when compared to HCV-infected animals[53], indicate the ability of HEV to modulate the interferon response. Unlike other hepatotropic RNA viruses (such as HAV and HCV), HEV does not cleave MAVS or degrade other host proteins engaged in signalling downstream of PRR, thereby enabling a sustained type III interferon response in persistently infected cells[46,55]. The induction of a persistent type III interferon response can hamper cellular responses to type I interferons, particularly IFNα[83,84]. In comparative studies based on subgenomic replicon RNAs (self-amplifying, subgenomic viral RNAs that cannot form infectious particles), HEV replication in vitro was further found to be more resistant to exogenous treatment with either IFNα or IFNλ than HCV[46,85,86]. In agreement with this observation, IFNα treatment has been found to exert moderate but delayed antiviral activity against HEV in patients[86–88], whereas patients with chronic HCV who respond to IFNα therapy often experience a rapid and sharp reduction in viral loads[89]. In addition, other interferons, such as IFNβ, IFNγ, and λ1, λ2 and λ3, displayed negligible effects on HEV replication[86].

Owing to its virological efficacy, safety and tolerability, interferon-free direct-acting antiviral therapy has now emerged as the standard treatment for HCV, surpassing the therapeutic utilization of interferon[90]. Although comparisons of HCV genomes have revealed genomic regions that determine resistance to interferon treatment[91], molecular determinants that modulate HEV interferon sensitivity have not yet been identified. On the other hand, pharmacological inhibition of JAK1, a key kinase mediating activation of the interferon signalling network, strongly facilitated HEV but not HCV infection in cell culture models[80,86]. This disparity between basal and therapeutic interferon sensitivity in vitro might also reflect the distinct incidence of chronic disease upon exposure to HEV or HCV. Chronic hepatitis E is mainly reported in immunocompromised patients, whereas between 55% and 85% of individuals infected with HCV[92], including immunocompetent individuals, develop chronic hepatitis C. Hence, immunosuppressants are considered to be a key factor in the development of chronic hepatitis E. The fact that approximately one-third of patients with chronic HEV achieve viral clearance after reduction of immune suppression highlights the importance of endogenous immune signalling to the control of HEV infectivity[15] (Fig. 2a). Variations in genes related to type III interferons have been linked to both natural and treatment-induced clearance of HCV[93–95]. Currently, it remains unknown whether similar type III interferon polymorphisms are associated with clearance versus persistence of HEV upon withdrawal of immunosuppression.

Immunosuppressive therapy based on tacrolimus (rather than cyclosporin (CsA)) has been described as a strong predictive factor for the development of chronic hepatitis E, potentially due to a greater immunosuppressive effect of tacrolimus compared with CsA[96,97]. Tacrolimus and CsA are calcineurin antagonists that are used widely as T cell immunosuppressants and exert their immunosuppressive effects by reducing the transcriptional activity of nuclear factor of activated T cells, resulting in reduced IL-2 production and IL-2 receptor expression, leading to a reduction in T cell activation[98]. CsA has also been observed to boost HEV replication by targeting cyclophilins A and B in human HuH-7 liver cells[99]. Hence, different immunosuppressants could further differentially affect the course of an HEV infection independently of their effects on the immune system[100]. In this context, the ability of HEV to replicate in the presence of interferons might facilitate its persistence if T cell function is impaired.

Various in vitro studies using overexpression systems and recombinant strains suggest that HEV dampens PRR activation and interferon signalling and reduces the effects of selected ISGs (reviewed previously[51]) (Fig. 2b). Similar to other hepatitis viruses, HEV also impedes PRR sensing via MDA5 and RIG-I, indicating a crucial role in the disruption of interferon production to facilitate hepatotropic infection[51]. Particularly, HEV ORF1 products, including the X and putative PCP domain, were observed to inhibit the type I interferon response by blocking RIG-I and TBK1 ubiquitination and IRF3 phosphorylation in vitro[101,102]. Overexpression of either methyltransferase or putative PCP domain inhibited interferon induction by interfering with aspects of MDA5 signalling, including NF-κB phosphorylation[103–106]. The ORF3 protein was found to inhibit IFNα-induced phosphorylation of STAT1 and impair IFNα-stimulated gene expression[107]. Moreover, ORF3 expression inhibited inflammatory NF-κB signalling[108] and activation of JAK–STAT and JNK–MAPK pathways, potentially by suppressing expression of TLR3 and TLR7 (ref. 109). Further, overexpression of ORF3 in THP-1 macrophages has been observed to inhibit the secretion of inflammatory cytokines by suppressing activation of the NF-κB pathway[110]. Given that HEV is capable of replication within macrophages[111], ORF3 might contribute to reduced immune clearance in immunocompromised patients. Likewise, the capsid protein ORF2 antagonized NF-κB signalling[112,113] and interferon induction by either blocking TBK1-mediated phosphorylation and dissociation of IRF3 from MAVS[114] or by inhibiting RIG-I and TLR adapters[115]. Collectively, these mechanisms might shape a cellular environment that favours HEV infection. Unfortunately, HEV is notoriously difficult to culture in vitro and only a few cell culture systems have been established[16,116]. Furthermore, processing of the ORF1 polyprotein remains a subject of debate[117]. However, numerous studies rely on data derived from the overexpression of individual ORF1 fragments, possibly in settings divergent from their real-life contexts. Hence, the clinical relevance of these observations as well as whether they reflect wild-type HEV characteristics remains to be determined.

Innate immune cells during HEV infection

The innate antiviral immune response also involves natural killer cells, dendritic cells, granulocytes, monocytes, macrophages and innate lymphoid cells. Hepatic macrophages have a central role in maintaining homeostasis within the liver and in initiating inflammatory responses to pathogens[118]. Macrophages can sense pathogens via different PRRs, including RIG-I, TLRs and NOD-like receptors (NLRs). NLRs can form large cytoplasmic complexes called inflammasomes that link sensing of PAMPs to proteolytic activation of pro-inflammatory cytokines, in particular IL-1β[119]. HEV infection has been observed to robustly trigger NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation in primary human macrophages and macrophage cell lines, inhibiting an interferon response, suggesting a potential mechanism of HEV-induced hyperinflammation and liver damage[75]. In addition, patients with HEV1 infection displayed increased levels of macrophages and dendritic cells, especially in patients with acute liver failure (ALF)[41]. Interestingly, the same study also reported functional impairments of macrophages in pregnant women with HEV1 infection with ALF, including reduced phagocytic activity and defective TLR signalling[41]. In addition to antiviral treatment, pharmacological strategies that modulate the functionality of monocytes and macrophages might provide an avenue for targeting HEV and simultaneously reducing pathological inflammation[75].

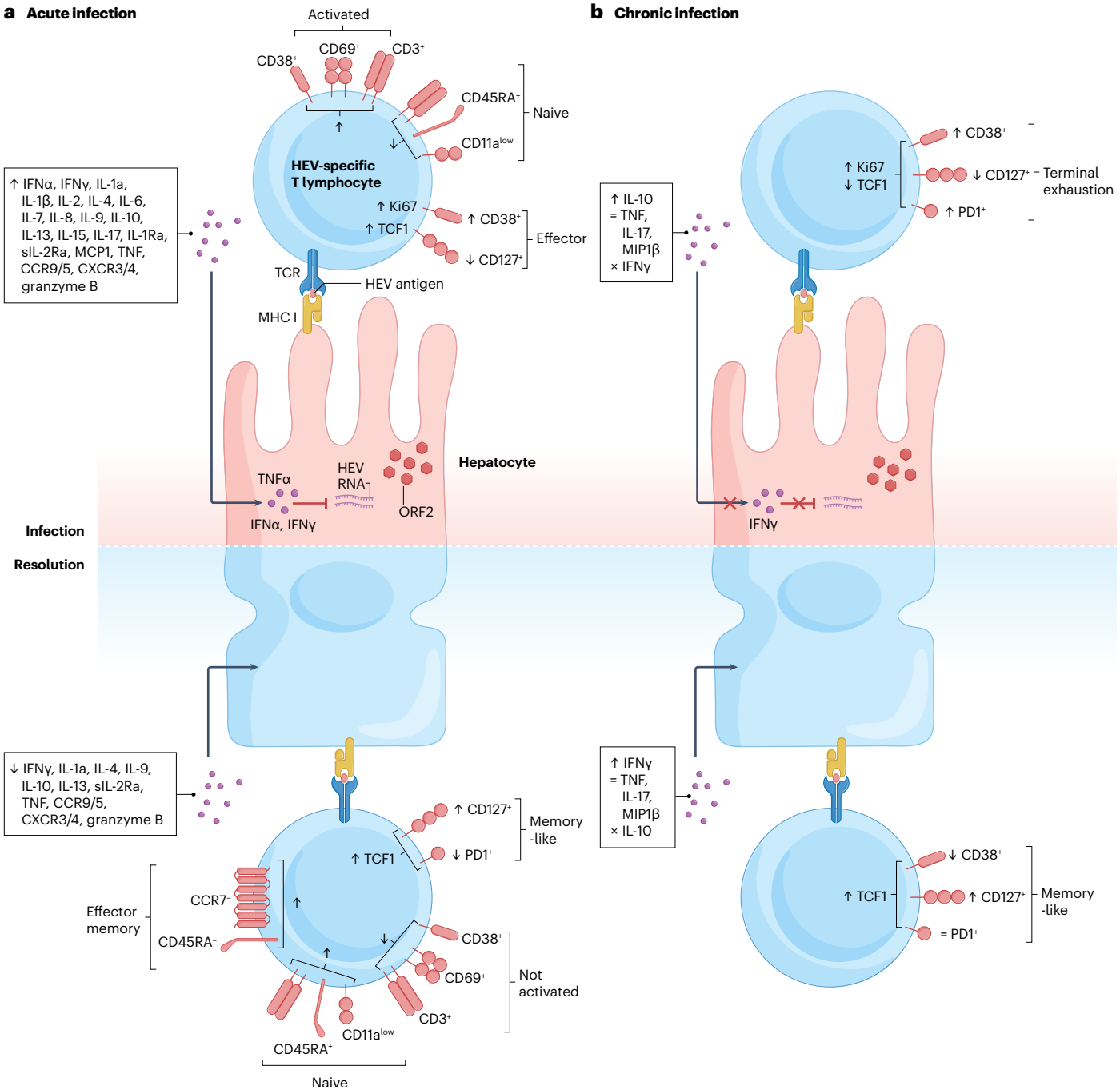
Natural killer cells constitute the main population of lymphocytes residing in the liver and are characterized by the expression of CD56 and CD16 as well as by the lack of CD3 (refs. 120,121). Natural killer cells are important in early innate immune responses and have a substantial role in both the pathogenesis of and defence against viral hepatitis via direct cytotoxicity and release of antiviral cytokines, respectively[122]. Upon activation, natural killer cells can mediate innate cytotoxic activity against HEV-infected hepatocytes but might also contribute to an inflammatory environment that leads to hepatocellular injury. Analysis of PBMCs from patients with acute hepatitis E demonstrated lower frequencies of natural killer cells compared with healthy individuals as controls, implying mobilization towards liver tissues[123,124]. In line with this, CD56 (a natural killer cell marker) and granzyme B (a natural killer cell activation marker) were substantially increased in liver tissues from patients with HEV-related ALF compared with ALF induced by other hepatitis viruses (A, B, C) and liver biopsy samples from control patients[125]. However, the levels of $CD56^+$ natural killer cells were low across all groups, and the individuals included in this study were in the terminal stage of the disease. Increased amounts of CD69, an early marker of lymphocyte activation, have also been detected in immunocompromised transplant recipients with acute hepatitis E[19]. These alterations in natural killer cell number and activation status ($CD56^+CD69^+$) reverted in patients with acute hepatitis E during convalescence to comparable values observed for healthy controls[123], implying a role during HEV pathogenesis. Increased CD69 expression has also been detected in γδ T cells in solid organ transplant recipients with acute HEV infection[19] and linked to IL-10 expression in patients with acute hepatitis E[126]. γδ T cells are classified by the expression of γ and δ TCR chains, and they both participate in liver protection while also contributing to lymphocyte-mediated organ damage[127]. However, other than these observations, the precise role of natural killer cells and other innate lymphoid cells during HEV infection and pathogenesis remains poorly defined. Neither the phenotype nor the function of these cell populations have been studied in relation to disease activity or outcomes of infection. Interestingly, phenotypic and functional alterations in natural killer cells, including decreased production of antiviral cytokines, have been observed in patients with HBV, HCV and HDV infections[122,128]. This raises interesting questions regarding whether there are similar phenotypic and functional differences within natural killer cell populations in patients with acute versus chronic HEV, and how viral infection is influenced by host genetics[129] and viral evolution[130].

Features of adaptive immunity to HEV

T cell-mediated adaptive immunity to HEV

T cells have a central role in the specific, targeted elimination of hepatotropic virus infections[131,132]. Upon recognizing viral antigens, T cells undergo activation, rapidly proliferate and differentiate into effector T cells, including cytotoxic T cells ($CD8^+$) that specialize in destroying infected cells and helper T cells ($CD4^+$) that support the activation and coordination of other immune cells necessary for viral clearance. The subsequent formation of long-lasting memory T cells ensures a rapid and robust response upon re-exposure to the same virus and is essential for establishing immunological 'memory'[133].

Evidence of T cell involvement in HEV clearance[18,134–136] and liver pathogenesis[125,134,137–141] during HEV infection has been reported, with initial studies in the early to late 2000s focusing on acute HEV1 infection[134,137–139,142,143]. Interest in T cell-mediated immune responses was later reignited when HEV chronicity was discovered in immunocompromised solid organ transplant recipients[18], prompting further investigation into T cell responses to HEV3 in both acute[19,135,140,144] and chronic infections[135,136,144,145].



Patients with acute hepatitis E mount robust CD4$^+$ T cell[134,146] and CD8$^+$ T cell responses[147] (Fig. 3a). These responses are predominantly directed against the viral capsid ORF2 protein[19,135,136,138,140,142,145,146] rather than the ORF3 protein[19,135,136,138,140,146]. However, T cell responses also target overlapping peptides within HEV ORF1 as long as T cell targets are mapped predominantly to well-conserved genomic regions[135,145]. Notably, T cell epitope mapping efforts have identified several immunodominant regions within HEV ORF2 that are associated with substantial proliferation (spanning amino acids 73–156, 289–372, 361–444 and 505–588)[146] and IFNγ-producing T cell responses (spanning amino acids 181–249 and 301–489)[138]. Notably, most lymphoproliferative immune responses to different regions of the ORF2 protein showed no association with specific HLA alleles, except for the HLA-DRB1 allele 010X, which was associated with a peptide pool corresponding to amino acids 289–372 of the HEV ORF2 protein[146].

Moreover, a high frequency of polyfunctional CD4$^+$ and CD8$^+$ T cells[138,147] are present in the peripheral blood and are capable of specifically cross-reacting with HEV genotypes 1 and 3 in humans[140]. As a result of lymphocyte activation, the number of naive CD3$^+$CD45RA$^+$ T cells declines[147], and effector T cells expressing and secreting

Fig. 3 | HEV-specific T cell and cytokine response to acute and chronic infection. a, In patients with acute hepatitis E, hepatitis E virus (HEV)-specific $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells are expanded, exhibiting different characteristics of activated and polyfunctional T cell immune responses. Consistent with the identification of HEV-specific IFNγ-producing cells[138,139], high levels of IFNγ (a hallmark cytokine for effector T cell responses) are detected in the supernatant of antigen-stimulated peripheral blood mononuclear cells, accompanied by the upregulation of IFNγ mRNA transcripts in HEV antigen-stimulated peripheral blood mononuclear cells[134]. Furthermore, following HEV antigen stimulation, there is an elevated release of other cytokines implicated in liver pathogenesis (IL-1a, soluble IL-2 receptor-α (sIL-2Ra))[139], coupled with an upregulation (compared with patients in the resolving phase) of cytokine mRNA transcripts associated with T helper type 1 (T_H1) or T_H2 differentiation (IFNγ, IL-2, TNF (T_H1); IL-4, IL-10 (T_H2)). In liver biopsy samples from patients with acute hepatitis but not in healthy individuals, granzyme B, an effector molecule produced by activated $CD8^+$ T cells, is also detected[125]. Moreover, patients with hepatitis E exhibit heightened levels of serum IL-1β and, during the viraemic phase, additional pro-inflammatory and anti-inflammatory cytokines (IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-15, IL-17, IL-1Ra, MCP1, TNF and IFNγ) are detectable in plasma. Among these, IL-13 and IL-9 notably decrease following viral clearance[140]. Cell-mediated immune responses are associated with elevated frequencies of $CD3^+$ T cells expressing markers indicative of antigen recognition ($CD38^+$) and early T cell activation ($CD69^+$) in the blood of patients with acute hepatitis E compared with healthy controls and patients in the resolving phase[147]. Concurrently, there is a notable reduction in the population of naive $CD3^+CD45RA^+$ T cells among patients with acute HEV. Furthermore, both $CD4^+$ and $CD8^+$ naive T cells exhibit a significantly higher proportion of the CD11a integrin (a marker for T cell recruitment to target tissue) in the resolved phase as opposed to the acute phase[147]. Transcript analysis of tissue-specific homing receptors also revealed increased expression of such receptors in patients with acute infection (CCR9 and CCR5) or those in the resolving phase (CCR9, CCR5, CXCR3 and CXCR4)[147]. A potent polyfunctional effector $CD8^+$ T cell response (high expression of the activation marker CD38, the proliferation marker Ki67, granzyme B and the transcription factor Tbet) is formed. Upon resolution, expression of CD38 and Ki67 is lost, giving rise to a memory response, evident through the expression of CD127 and T cell factor 1 (TCF1), as well as lower expression of granzyme B and PD1 on HEV-specific $CD8^+$ T cells[145]. The memory cell pool is then largely composed of the $CD45RA^-CCR7^-$ subset, featuring few $CCR7^+$ central memory $CD8^+$ T cells, suggestive of a robust memory response after resolution[145]. **b**, Less is known about the cellular immune response to chronic HEV. Current data suggest that HEV-specific T cell responses are primarily monofunctional and attenuated in comparison to acute infection. These T cell responses are characterized by heightened expression of CD38 and PD1, along with predominantly elevated levels of Ki67, while exhibiting diminished levels of CD127 and TCF1 — a characteristic profile associated with terminally exhausted $CD8^+$ T cells[145]. After resolution of chronic infection, a sufficient reservoir of memory-like $CD8^+$ T cells emerges, characterized by moderate PD1 expression, the presence of CD127 and TCF1, and a reduction in CD38 expression[145]. HEV-specific cytokine responses in patients with chronic HEV are notably characterized by elevated levels of IL-10 (T_H2 response) and a lack of IFNγ production (T_H1 response), as discerned from cell culture supernatants following peptide pool stimulation. Conversely, an absence of IL-10 and a strong IFNγ response is observed in patients with resolved HEV infection. Noteworthy production of IL-17 (T_H17 response), macrophage inflammatory protein (MIP1β) and TNF can be detected in both chronic and resolved patient groups[136].

IFNγ[134,138–140,145] emerge in PBMCs of patients with acute infection. The $CD45RA^+CD11a^{high}$ T cell subset also expands during acute infection and expresses and displays several homing receptors (such as CCR9), indicating functional recruitment from the periphery to the liver and the site of inflammation[147]. In agreement, lymphocyte infiltration of predominantly activated $CD8^+$ T cells containing granzymes can be observed in liver biopsy samples from patients with HEV-induced ALF[125,137]. After viral clearance, HEV-specific $CD8^+$ T cells further differentiate into an effector memory-like phenotype ($CD45RA^-CCR7^-$) and become more monofunctional (monoproducers of MIP1β) in humans[135]. In patients with resolved HEV infection, T cell levels initially decline but then reach a plateau that remains stable for up to 4–12 years[135,140,145], suggesting a strong and sufficient memory response with the potential to protect against re-infection. In immunocompetent older population cohorts, adverse clinical outcomes upon HEV3 infection, ranging from asymptomatic to acute icteric hepatitis and severe liver damage in one-third of patients[148–150], have been associated with host T cell-mediated immune responses[141]. In symptomatic patients, this response is characterized by a robust expansion of highly activated effector memory $CD8^+$ T cells, which is often linked to early T cell exhaustion[141]. In addition, there is an increase in inflammatory chemokines (CXCL9 and CXCL10), which are involved in recruiting highly cytotoxic effector memory cells[141]. Loss of polyfunctional T helper 1 (T_H1) cell cytokine production (such as IL-2, TNF and IFNγ) and commitment to T_H2 cells (IL-4, IL-5 and IL-10) is also a feature of T cell-mediated immune responses in older symptomatic patients. Interestingly, this bias towards a T_H2 profile can also be observed in pregnant women with acute HEV[151], and during HEV-related acute liver failure[152] and chronic HEV[136], suggesting a close correlation between the T_H1 and T_H2 response, disease aggravation, and clinical outcome. This effector memory-biased immune response reverts to homeostasis after viral clearance and disease resolution[141].

In contrast to patients with acute hepatitis E, patients with chronic hepatitis E lack a robust and multi-specific $CD4^+$ and $CD8^+$ T cell response specific to HEV and fail to secrete antiviral cytokines such as IFNγ[18,19,135,136] (Fig. 3b). In these patients, epitope-specific T cells show signs of activation, as indicated by CD38 and Ki67 expression, but predominantly exhibit a $CD127^{low}PD1^+$ phenotype, which is characteristic of terminally exhausted $CD8^+$ T cells[145]. Similar to observations in chronic hepatitis B or C, T cell exhaustion during chronic HEV infection might be attributable to persistent exposure to viral antigens such as secreted ORF2 proteins[145,153]. Following resolution of chronic hepatitis E (that is, through a reduction in immunosuppression and/or ribavirin treatment), robust HEV-specific T cell responses[136] and high levels of IFNγ expression[19,136] become rapidly detectable, typically within 4–8 weeks after viral clearance[136]. During this phase, $CD8^+$ T cells acquire a memory-like phenotype characterized by moderate PD1 expression, CD127 positivity and TCF1 expression. The majority of these cells no longer express the activation marker CD38, indicating the persistence of a substantial reservoir of memory-like $CD8^+$ T cells after viral elimination[145].

Reinvigoration of functional $CD4^+$ and $CD8^+$ proliferative responses is partially possible in vitro by blocking the co-inhibitory receptors PD1 or CTLA4 but might depend on interindividual variability and intraindividual differences between $CD4^+$ and $CD8^+$ T cell responses to PD1 or CTLA4 antibody treatment[136]. In addition, it is important to note that checkpoint inhibition, such as blocking the PD1 pathway, might not be a suitable treatment strategy in transplant recipients following ribavirin treatment failure as it might induce transplant rejection[145].

As T cells gradually progress towards a terminally exhausted state through different stages[154], several other potential approaches and targets to reinvigorate terminally exhausted T cells are conceivable.

These approaches could include drugs that target other intrinsic T cell pathways. For example, targeting transcription factors, such as TOX[155–157] or NR4A[158], as well as molecules such as TGFβ[159] or the E3 ubiquitin-protein ligase CBL-B[160] might have the potential to promote T cell function. Furthermore, strategies for targeting terminally exhausted T cells might also involve drugs that target hypoxia or co-stimulation pathways[161]. Gaining a deeper understanding of the mechanisms of exhaustion during viral infection remains a key aspect of identifying novel targets to counter T cell exhaustion.

Owing to these observations, reinvigorating or inducing HEV-specific T cell responses by redirected T cells could be a viable strategy to effectively treat chronic hepatitis E in immunosuppressed patients upon failure of ribavirin therapy. Intriguingly, a screening of HEV-specific $CD8^+$ T cell immune responses in HLA-$A2^+$ patients with acute hepatitis E identified two HEV-specific $CD8^+$ T cell epitopes located at the RNA helicase (HEV-1116.B3) and RNA-dependent RNA polymerase (HEV-1527.A2)[162]. Subsequently, next-generation sequencing of TCR repertoires identified HEV-specific TCRs that were redirected into lymphocytes isolated from patients with chronic hepatitis E. TCR-redirected T cells induced polyfunctional HEV-specific immunity, recognition of virus-specific epitopes and mediation of target cell killing in vitro[162]. A follow-up investigation that explored the concern that these antigen-specific T cells might cross-recognize self-peptides (a potential cause of autoimmunity) found that one of the identified TCRs could indeed cross-recognize the apoptosis-related epitope non-muscle myosin heavy chain 9 (ref. 163). However, when T cells were stimulated in the presence of both HEV-1527 and MYH9-478 peptides, T cells were selectively activated by the HEV-1527 peptide and remained non-responsive to the MYH9-478 peptide. This observation of cross-recognition but lack of functional cross-activation is further associated with higher avidity of TCR towards HEV-1527 than MYH9-478. Regardless, the researchers note that they included only a limited scope of self-antigens in their in vitro screening and that, before advancing to immunotherapy, careful assessment of auto-reactivity to other self-peptides is indispensable to minimize off-target toxicity[163]. In addition, these initial landmark studies on redirected T cell therapy are based on in vitro observations and have so far been restricted to individuals carrying the HLA-A*02 allele. Thus, in vivo efficacy and broader clinical trials are needed to fully evaluate the potential of the approach for a wider population of patients with diverse HLA backgrounds. Individual T cell clones exhibit a high degree of specificity in their recognition of epitopes, which suggests that viral mutations might lead to the selection of escape variants to evade immune pressure. The occurrence of T cell escape mutations has generally been observed in patients with HBV, HIV and HCV[164–166]. Interestingly, evidence for a mutational viral escape epitope ($A*01/ORF2_{389–397}$ (leucine to valine substitution at position 395)) that contributed to HEV-specific $CD8^+$ T cell failure emerged in one patient with chronic infection[145]. This variant was no longer able to stimulate the expansion of PBMCs and was associated with a $PD1^{low}CD38^-$ phenotype (indicating loss of antigen recognition). T cell activation, as indicated by the downregulation and internalization of the TCR as well as increased granzyme B expression, was exclusively observed upon re-stimulation of consensus peptide-expanded $CD8^+$ T cells with the consensus peptide but not with the variant peptide, indicating loss of activation by the escape variant[145]. As a result, personalized approaches, such as the generation and application of TCR-redirected $CD8^+$ T cells, might be required for viral clearance in non-responsive patients. However, before opting for this labour-intensive and cost-intensive procedure, it is crucial to carefully evaluate the potential of mutational viral escape.

Quantitative and qualitative analysis of complex T cell repertoires and T cell specificity and functionality to viruses are essential for understanding HEV pathogenesis and disease outcome. For HEV, functional assays that measure cytokine secretion (such as HEV-specific IFNγ enzyme-linked immunosorbent spot, enzyme-linked immunosorbent assay or intracellular cytokine staining) have been widely emp loyed[134,136,139,142,144,151]. Importantly, most of the data presented here combined several of these techniques to study HEV-specific T cell responses[134,135,138]. The latest studies in particular notably integrated both functional assays and those assessing T cell specificity (such as MHC tetramer staining) to achieve a more comprehensive analysis[145]. Furthermore, these studies utilize direct ex vivo stimulation, avoiding previous in vitro amplification of T cells, and thereby providing a more precise representation of the in vivo immune response[135,145].

In the future, whole-blood peptide-stimulation assays might help to avoid the need for immediate processing and loss of functional antigen-presenting cells due to processing and cryopreservation. Machine learning-based T cell receptor repertoire analysis (reviewed previously[167]) might enable prediction of TCR-binding specificity[168] and identification of HEV-specific TCR sequences[169].

B cell and antibody responses

B cells are specialized in producing and secreting HEV-specific antibodies, which serve as the first line of defence against re-infection[170]. In the peripheral blood of individuals experiencing fulminant and uncomplicated hepatitis E, $CD19^+$ B cells targeting ORF2 and ORF3 antigens undergo expansion and generate IgG[171] (Box 1). Notably, the proportion of HEV-specific IgG-secreting B cells is elevated in patients with fulminant hepatitis E compared with those with uncomplicated HEV infection[171]. Furthermore, higher frequencies of functional immature or transitional $CD19^+CD24^{hi}CD38^{hi}$ B cells (also known as B regulatory cells) have been observed in individuals with acute hepatitis E[172]. These B cells exhibit the potential for modulating IFNγ-mediated T cell responses in hepatitis E through IL-10-induced regulatory activities[172].

The humoral immune response to acute HEV infections is characterized by an early and transient appearance of IgM against the ORF2 capsid protein (Box 1 and Supplementary Figure 1a,b), which coincides with the detection of IgA antibodies[173] and viraemia[174] (Fig. 2a). Both the detection of IgM and IgA have therefore been utilized for the diagnosis of acute primary HEV infections. IgM immune responses remain at high levels for 8 weeks after the time of clinical presentation[175] (Fig. 2a). Subsequently, IgM antibodies rapidly wane during the convalescence period[176,177] and become undetectable in most patients between 3 and 8 months after disease onset[175,178]. During the late acute phase of illness, the IgM response transitions to a high-titre, high-avidity IgG response[170,179]. Peak ORF2-specific IgG levels are observed approximately 4 weeks after the onset of symptoms in patients with acute hepatitis E and are maintained at high levels for more than 1 year[175]. IgG antibody titres decline over time but persist in low to moderate levels for at least 20 months and up to 14 years[170,176,180,181]. Although most of the aforementioned observations pertain to HEV1 and HEV4 humoral immune responses, studies in asymptomatic, transfused or zoonotically transmitted patients have established that IgM and IgG antibody durability and kinetics are similar in acute resolving HEV3 infections[179,182–184]. Among patients with chronic hepatitis E, high individual variability in the duration and dynamics of humoral immune responses is observed. In solid organ transplant recipients with

Box 1 | Immunological characteristics of the ORF2 capsid protein

Hepatitis E virus (HEV) virions are *T=3* icosahedral spherical particles with a diameter of 270–300 Å (refs. 14,232) (Supplementary Figure 1a). These particles form through the assembly of 180 copies of the ORF2 capsid protein[232]. ORF2 proteins are major antigens and determinants of long-lived antibody-mediated responses and protective immunity against HEV[233]. Notably, both a capsid protein variant ($ORF2^C$) and a secreted glycosylated variant ($ORF2^S$) are expressed in patient sera and supernatants of HEV-infected cell cultures[153,234]. Unlike $ORF2^C$, $ORF2^S$ is not associated with virus particles or receptor-mediated uptake by cells; however, it is implicated in inhibiting antibody-mediated neutralization and is suggested to have a role in immune evasion[153,234]. Notably, ORF2 single-nucleotide variants with immune decoy functions were found to circulate in patients with HEV treated with ribavirin[235]. Structurally, monomeric ORF2 proteins are composed of three domains: a shell domain (S domain and amino acids (aa) 118–317), a middle domain (M domain and aa 318–451), and a protrusion domain (E2s or P domain and aa 452–606)[236–239] (Supplementary Figure 1b). The P domain is composed of homodimeric subunits (domain E2 aa 394–606; domain E2s aa 455–602)[226,240] that form protrusions that participate in cell binding and harbours epitopes for antibody neutralization[236,240–242]. When N-truncated and C-terminally cleaved versions of the ORF2 capsid protein, spanning at least aa 112–608, are expressed in a recombinant baculovirus system in insect cells, empty virus-like particles self-assemble from 60 units of 50 kDa ORF2 capsid proteins. These capsomers configurate into *T=1* surface lattices with protruding dimers located at each of the icosahedral twofold axes[35,243]. Although these particles are smaller in diameter than the native HEV particles, they retain systemic antigenicity in vivo[244] and demonstrate antibody reactivity to the native HEV antigen[245]. HEV-like particles have thus been used for the development of safe and cost-effective virus-like particle-based vaccines and diagnostic tests[222]. HEV-like particles have also been proposed as vaccine carriers for foreign epitopes[246]. Specifically, the C-terminal P domain and connecting region between the S and P domains have been shown to be broadly reactive with patient sera and have a crucial role in anti-HEV antibody binding[241,247–250]. Several immunodominant conformational and linear epitopes have been identified[220,238,242,251–254], which are commonly targeted for cross-neutralization of different HEV genotypes[220,254–256]. The only approved HEV vaccine, HEV 239, is a 239 amino acid-long recombinant HEV peptide spanning aa 368–606 of the HEV1 viral capsid protein (Supplementary Figure 1c); upon purification, HEV 239 peptides form homodimers with a size of 23 nm (highlighted in red/grey dashed structure)[257]. Other vaccine candidates (rHEV and p179) are currently being developed in various clinical phases[216,222].

chronic HEV3 infection, both persistent[63,185–188] and absent[186,189] anti-HEV IgM and IgG responses have been reported. In patients with persistent anti-HEV IgM and IgG responses, seroconversion is frequently delayed[186,187,190], which, along with the absence of seroconversion, is suggested to correlate with intense immunosuppression through regimens of calcineurin inhibitors, steroids and anti-proliferative drugs (drugs that decrease the synthesis of antibodies[191,192] and inhibit the cell-cycle progression and differentiation of human B cells[193]) in patients with chronic infection[189,190,194].

Understanding the duration and protective potential of the HEV-specific antibody response is crucial in determining whether individuals who have recovered from HEV or received vaccination can mount an effective immune response upon HEV re-exposure. The current body of evidence suggests that while active and passive immunization effectively prevents severe acute hepatitis, it might not universally confer sterilizing immunity or lifelong protection against subsequent HEV re-infections[180,195]. Notably, declining anti-HEV antibodies were observed in large proportions (~95%) of individuals with previous infection[180,196]. Over follow-up periods ranging from 1 to 22 years[180,197–199], seroreversion rates of around 21.7% (5/23 blood donors)[199], 22.6%[198] and up to 50% (9/18 individuals)[197] were detected within 22, 12 and 7 years after initial anti-HEV antibody detection, respectively, suggesting the possibility of re-infection. In cases in which previously seroconverted rhesus macaques were re-inoculated with homologous HEV, most animals remained protected from re-infection, while others experienced subclinical HEV viraemia marked by reduced virus shedding, lowered HEV RNA levels, absence of IgM anti-HEV antibody responses and elevation of alanine aminotransferase levels[200]. Similar observations have been made in primates challenged with a heterologous HEV isolate — derived either from the same or different host species — following primary inoculation[201–203]. This highlights the notion that previous HEV infection might offer cross-genotype and cross-host-species protection, albeit without achieving sterilizing immunity. Furthermore, the susceptibility and effective protection to HEV re-infection is tied to anti-HEV IgG titre and avidity[200,201]. Notably, pre-existing IgG antibody avidity exceeding 50%, in conjunction with elevated IgG titres, seems to provide protection against homologous re-infection[200,201], whereas low anti-HEV IgG levels have been associated with progression to chronicity in solid organ transplant recipients re-infected with HEV[204].

Thus far, data concerning the duration and progression of humoral immune responses have predominantly originated from early studies during epidemic and sporadic outbreak settings, often conducted before international anti-HEV antibody reference standards were established[205]. Therefore, it is important to acknowledge the need for caution when interpreting and comparing serological data given that these conclusions stem from studies that employed a range of antigens and testing kits with differing sensitivities and specificities and originating from various manufacturers and suppliers.

Prophylactic vaccination

Vaccination strategies against HEV are particularly relevant for high-risk groups, including pregnant women, immunocompromised patients and those with pre-existing liver disease[206]. Attempts to develop active immunization strategies have predominantly centred on protein-based vaccines, which aim to induce protective, neutralizing antibody responses. Notably, the only approved HEV vaccine is HEV 239 (Xiamen Innovax Biotech Co., Ltd., China). HEV 293 is commercially marketed as Hecolin since obtaining authorization in 2011 in China for individuals aged 16 and above who are at risk of HEV infection (NCT02189603)[207–209]. This highly efficacious vaccine is based on a truncated HEV1 recombinant ORF2 peptide (amino acids 368–606) consisting of 239 amino acids[210] (Box 1 and Supplementary Figure 1b,c). A large double-blind, randomized, placebo-controlled trial involving 112,604 participants from China aged 16–65 years provided compelling

evidence of cross-genotype protection induced by HEV-specific antibodies[208]. The vaccine exhibited notable efficacy in preventing symptomatic infections, particularly in regions with a predominant prevalence of HEV4. Over a 4.5-year follow-up assessment of efficacy, immunogenicity and safety, seven HEV infections were confirmed in participants who had received the HEV 239 vaccine, resulting in a vaccine efficacy of 86.8% (95% CI 71–94)[207]. A first-ever hepatitis E reactive mass vaccination campaign targeting 27,000 individuals aged 16–40 years (including pregnant women) in the internally displaced persons camp in Bentiu, South Sudan, was initiated in March 2022 in response to a HEV1 outbreak in the region. A vaccination coverage survey from 2024 demonstrated high self-reported coverage of at least one dose of Hecolin in the Bentiu camp community[211]. Passive surveillance found no severe adverse events following immunization[212]; however, data on vaccine effectiveness are currently not available.

Between 2001 and 2017, two other vaccines were investigated in clinical trials: rHEV and p179 (Box 1 and Supplementary Figure 1c). rHEV is produced in insect cells by recombinant baculoviruses and showed cross-genotype protective immunogenicity in a preclinical macaque study[213]. The protein-based vaccine, which expresses amino acids 112–607 of the capsid protein of HEV1 (Sar-55 strain), was also found to be well tolerated (apart from local pain at the injection site) and immunogenic in healthy volunteers from the USA[214] and Nepal[215]. A subsequent randomized, double-blind, placebo-controlled phase II study (NCT00287469) in 1,794 individuals from the Nepalese Army (mostly men) demonstrated efficacy of 95.5% for preventing HEV infection during a median follow-up of 804 days[216]. However, despite these positive results, this trial raised ethical concerns, and no further clinical testing of the vaccine has been conducted since[216–219]. Similar to HEV 239, p179, representing amino acids 439–617 of the HEV4 capsid protein, is expressed in *Escherichia coli*[220,221]. A randomized, open-label, parallel-controlled phase I clinical trial (120 study participants) showed safety and good tolerance in individuals aged between 16 and 65 years from the Jiangsu province of China[222]. Data on immunogenicity in humans are not yet available. A phase II study of the p179 vaccine is currently ongoing[222].

To date, routine use of an effective HEV vaccine has encountered several difficult challenges[223], including limited awareness about HEV and its vaccine among regulators and policymakers, uncertainties about vaccine supply, and a lack of information regarding permissible flexibility in dosing schedules and long-term effectiveness. In addition, there is a pressing need to further evaluate the efficacy and safety of existing HEV vaccines and vaccine candidates under development, particularly in vulnerable groups at risk (such as pregnant women, individuals under 16 years, and individuals immunocompromised due to HIV or medically induced immune suppression) and to achieve WHO prequalification and licensing outside China and Pakistan. If a commercially licensed vaccine for HEV becomes available worldwide, understanding the precise HEV burden in endemic areas, the potential cross-protection of the vaccine against all genotypes, and the possibility of its concomitant use with other vaccines would further assist in developing a vaccination policy[223,224].

Box 2 | HEV-specific immune responses: outstanding questions and challenges

- Which interferon-stimulated genes can restrict hepatitis E virus (HEV) infectivity within hepatocytes, and are there interferon-stimulated genes that potentially act as tissue-specific and/or species-specific restriction factors determining HEV tissue and/or host range?
- How does HEV persist despite a continuous interferon response?
- Which adaptive immune responses (humoral/cellular) are required to prevent HEV persistence?
- What is the major immune mechanism underlying HEV-induced liver damage?
- How long does protective immunity last following HEV infection?
- Screening and identification of T cell epitope viral escape: identification of viral escape by T cells is essential for developing strategies to overcome immune evasion and enhance the effectiveness of T cell-based therapies.
- To what extent does the HEV host-derived envelope protect the virus from neutralizing antibodies in vivo?
- Identification of antibodies that neutralize HEV pan-genotypically could serve as a potential future strategy for passive immunization.

Conclusions

Collective evidence from both human and animal studies has outlined an intricate balance of immune signalling events that control HEV infection. Immune dysfunction and inflammation seem to contribute to the different clinical manifestations of hepatitis E, including self-limiting acute viral hepatitis and ALF. Elucidating the pathological mechanisms that result from a dysregulated immune response, and how these relate to the virological characteristics (genotype, subtype, quasi-species) of patients, will be important to understand the different clinical manifestations of HEV. For example, although the immune response in healthy individuals usually clears HEV, virus exposure can eventually result in acute hepatitis and can carry forward into a chronic infection in immunocompromised patients. Withdrawal of immunosuppressive therapy only results in viral clearance in approximately 30% of patients with chronic hepatitis E. Thus far, the key factors of the immune responses required for successful HEV clearance and asymptomatic infection remain only partially understood (Box 2). However, it has become clear that cellular innate immunity alone does not provide adequate defence against HEV as chronic infection has frequently been reported in patients in whom the adaptive immune system is impaired.

In contrast, T cell immunity is considered to be of paramount importance in the resolution of enteric hepatitis virus infections but has also been linked to liver injury in hepatitis virus infection[84]. This role is also clearly supported in the context of HEV, as patients with acute, resolved HEV infection display robust and multi-specific $CD4^+$ and $CD8^+$ T cell responses, whereas narrow $CD8^+$ T cell responses have been observed in patients with chronic infection[145]. Hence, the breadth of the HEV-specific $CD8^+$ T cell response seems to be a crucial factor determining infection outcome. Similar to chronic HBV and HCV infections, prolonged exposure to HEV antigens seems to result in the functional exhaustion of $CD8^+$ T cells, rather than preventing the priming of HEV-specific $CD8^+$ T cells[84,145]. The resulting moderate but sustained selection pressure seems to further favour the emergence of viral escape mutations[145], which so far have only been observed upon treatment with ribavirin or the nucleotide analogue sofosbuvir[28–30]. Hence, reduced immunosuppression in combination with antiviral treatment should be started early in cases of persistent HEV infection as currently recommended by guidelines of the European Association for the Study of the Liver[12]. Likewise, it remains to be determined whether there are similar

phenotypic and functional differences within the innate immune cell populations of patients with acute versus chronic HEV.

Concerning the humoral immune response, it is widely acknowledged that neutralizing antibodies produced through either natural infection or vaccination have a significant ability to confer apparent protection against acute hepatitis. However, the extent to which antibodies contribute to the resolution of an acute infection as well as the frequency and circumstances under which antibody-mediated protection falters are yet to be conclusively ascertained. Efficient protective and/or therapeutic vaccines would be particularly desirable for individuals at risk such as pregnant women, immunosuppressed individuals and patients with pre-existing chronic liver disease. Understanding the mechanisms of divergent outcomes following HEV infection will further elucidate how viral immune modulation affects infection outcome and the mechanisms by which HEV resists interferon-mediated antiviral responses. Research to advance and build on these exciting discoveries and developments will no doubt reveal the key factors of immune responses required for successful HEV clearance. A deeper understanding of the detailed components of an effective immune response to HEV, both at the innate and adaptive levels, will be useful to guide the development of effective and cross-genotype protective vaccines and could further guide the development of individually tailored HEV therapies. For example, therapeutic targeting of immune mechanisms that contribute to HEV pathogenesis combined with antiviral therapy might provide a viable option for treating severe HEV infections. Finally, to advance our knowledge regarding the interplay between HEV and the immune system, more clinical data and authentic model systems are needed to adequately recapitulate and study the molecular and medical features of clinical HEV isolates.

Published online: 22 July 2024

References

1. Rein, D. B., Stevens, G. A., Theaker, J., Wittenborn, J. S. & Wiersma, S. T. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology* **55**, 988–997 (2012).
2. World Health Organization. *Hepatitis E: Fact Sheet* https://www.who.int/news-room/fact-sheets/detail/hepatitis-e (2021).
3. Li, P. et al. The global epidemiology of hepatitis E virus infection: a systematic review and meta-analysis. *Liver Int.* **40**, 1516–1528 (2020).
4. Khuroo, M. S. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am. J. Med.* **68**, 818–824 (1980).
5. Smith, D. B. et al. Update: proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). *J. Gen. Virol.* **101**, 692–698 (2020).
6. Nimgaonkar, I., Ding, Q., Schwartz, R. E. & Ploss, A. Hepatitis E virus: advances and challenges. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 96–110 (2018).
7. Gouttenoire, J. et al. Palmitoylation mediates membrane association of hepatitis E virus ORF3 protein and is required for infectious particle secretion. *PLoS Pathog.* **14**, e1007471 (2018).
8. Hartl, J., Wehmeyer, M. H. & Pischke, S. Acute hepatitis E: two sides of the same coin. *Viruses* **8**, 299 (2016).
9. Navaneethan, U., Al Mohajer, M. & Shata, M. T. Hepatitis E and pregnancy: understanding the pathogenesis. *Liver Int.* **28**, 1190–1199 (2008).
10. Kinast, V., Klöhn, M., Nocke, M. K., Todt, D. & Steinmann, E. Hepatitis E virus species barriers: seeking viral and host determinants. *Curr. Opin. Virol.* **56**, 101274 (2022).
11. Chauhan, A. et al. Hepatitis E virus transmission to a volunteer. *Lancet* **341**, 149–150 (1993).
12. European Association for the Study of the Liver. EASL clinical practice guidelines on hepatitis E virus infection. *J. Hepatol.* **68**, 1256–1271 (2018).
13. Huang, S.-J., Liu, X.-H., Zhang, J. & Ng, M.-H. Protective immunity against HEV. *Curr. Opin. Virol.* **5**, 1–6 (2014).
14. Balayan, M. S. et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* **20**, 23–31 (1983).
Identification of hepatitis E virus as a previously unknown non-A, non-B virus species, by immune electron microscopy.
15. Walker, C. M. Adaptive immune responses in hepatitis A virus and hepatitis E virus infections.*Cold Spring Harb. Perspect. Med.* **9**, a033472 (2019).
16. Todt, D. et al. Robust hepatitis E virus infection and transcriptional response in human hepatocytes. *Proc. Natl Acad. Sci. USA* **117**, 1731–1741 (2020).
17. Krain, L. J., Nelson, K. E. & Labrique, A. B. Host immune status and response to hepatitis E virus infection. *Clin. Microbiol. Rev.* **27**, 139–165 (2014).
18. Kamar, N. et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N. Engl. J. Med.* **358**, 811–817 (2008).
First description of chronic hepatitis E virus infection in organ-transplant recipients.
19. Abravanel, F. et al. Conventional and innate lymphocytes response at the acute phase of HEV infection in transplanted patients. *J. Infect.* **72**, 723–730 (2016).
20. Kamar, N. & Pischke, S. Acute and persistent hepatitis E virus genotype 3 and 4 infection: clinical features, pathogenesis, and treatment.*Cold Spring Harb. Perspect. Med.* **9**, a031872 (2019).
21. Gotlieb, N., Moradpour, D. & Shouval, D. Hepatitis A and E — differences and commonalities. *J. Hepatol.* **72**, 578–580 (2020).
22. Das, A. et al. Cell entry and release of quasi-enveloped human hepatitis viruses. *Nat. Rev. Microbiol.* **21**, 573–589 (2023).
23. von Felden, J. et al. The burden of hepatitis E among patients with haematological malignancies: a retrospective European cohort study. *J. Hepatol.* **71**, 465–472 (2019).
24. Dalton, H. R., Bendall, R. P., Keane, F. E., Tedder, R. S. & Ijaz, S. Persistent carriage of hepatitis E virus in patients with HIV infection. *N. Engl. J. Med.* **361**, 1025–1027 (2009).
25. Dalton, H. R. et al. Hepatitis E virus and neurological injury. *Nat. Rev. Neurol.* **12**, 77–85 (2016).
26. Kamar, N., Marion, O., Abravanel, F., Izopet, J. & Dalton, H. R. Extrahepatic manifestations of hepatitis E virus. *Liver Int.* **36**, 467–472 (2016).
27. Kinast, V., Burkard, T. L., Todt, D. & Steinmann, E. Hepatitis E virus drug development. *Viruses* **11**, 485 (2019).
28. Debing, Y. et al. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. *Gastroenterology* **147**, 1008–11.e7 (2014).
29. Gömer, A. et al. Emergence of resistance-associated variants during sofosbuvir treatment in chronically infected hepatitis E patients. *Hepatology*. **78**, 1882–1895 (2023).
30. Todt, D. et al. In vivo evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. *Gut* **65**, 1733–1743 (2016).
31. Aggarwal, R. & Jameel, S. Hepatitis E. *Hepatology* **54**, 2218–2226 (2011).
32. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **124**, 783–801 (2006).
33. Feng, Z. & Lemon, S. M. Innate immunity to enteric hepatitis viruses.*Cold Spring Harb. Perspect. Med.* **9**, a033464 (2019).
34. Thompson, M. R., Kaminski, J. J., Kurt-Jones, E. A. & Fitzgerald, K. A. Pattern recognition receptors and the innate immune response to viral infection. *Viruses* **3**, 920–940 (2011).
35. Li, K., Chen, Z., Kato, N., Gale, M. & Lemon, S. M. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-β production in hepatocytes. *J. Biol. Chem.* **280**, 16739–16747 (2005).
36. Devhare, P. B., Chatterjee, S. N., Arankalle, V. A. & Lole, K. S. Analysis of antiviral response in human epithelial cells infected with hepatitis E virus. *PLoS One* **8**, e63793 (2013).
37. Choi, Y. H., Zhang, X., Tran, C. & Skinner, B. Expression profiles of host immune response-related genes against HEV genotype 3 and genotype 1 infections in rhesus macaques. *J. Viral Hepat.* **25**, 986–995 (2018).
38. Lee, M. S. & Kim, Y.-J. Signaling pathways downstream of pattern-recognition receptors and their cross talk. *Annu. Rev. Biochem.* **76**, 447–480 (2007).
39. Devhare, P. B., Desai, S. & Lole, K. S. Innate immune responses in human hepatocyte-derived cell lines alter genotype 1 hepatitis E virus replication efficiencies. *Sci. Rep.* **6**, 26827 (2016).
40. Majumdar, M., Ratho, R. K., Chawla, Y. & Singh, M. P. Role of TLR gene expression and cytokine profiling in the immunopathogenesis of viral hepatitis E. *J. Clin. Virol.* **73**, 8–13 (2015).
41. Sehgal, R. et al. Impaired monocyte-macrophage functions and defective Toll-like receptor signaling in hepatitis E virus-infected pregnant women with acute liver failure. *Hepatology* **62**, 1683–1696 (2015).
42. Arya, R. P., Mishra, N., Biswas, K. & Arankalle, V. A. Association of Toll-like receptor 4 polymorphism with hepatitis E virus-infected Indian patients. *J. Viral Hepat.* **25**, 1617–1623 (2018).
43. Xu, L. et al. RIG-I is a key antiviral interferon-stimulated gene against hepatitis E virus regardless of interferon production. *Hepatology* **65**, 1823–1839 (2017).
44. Li, Y. et al. MDA5 against enteric viruses through induction of interferon-like response partially via the JAK-STAT cascade. *Antivir. Res.* **176**, 104743 (2020).
45. Wang, W. et al. The RNA genome of hepatitis E virus robustly triggers an antiviral interferon response. *Hepatology* **67**, 2096–2112 (2018).
46. Yin, X. et al. Hepatitis E virus persists in the presence of a type III interferon response. *PLoS Pathog.* **13**, e1006417 (2017).
47. Rehwinkel, J. & Gack, M. U. RIG-I-like receptors: their regulation and roles in RNA sensing. *Nat. Rev. Immunol.* **20**, 537–551 (2020).
48. Sooryanarain, H., Heffron, C. L. & Meng, X.-J. The U-rich untranslated region of the hepatitis E virus induces differential type I and type III interferon responses in a host cell-dependent manner. *mBio* **11**, e03103-19 (2020).
49. Uzri, D. & Gehrke, L. Nucleotide sequences and modifications that determine RIG-I/RNA binding and signaling activities. *J. Virol.* **83**, 4174–4184 (2009).
50. Saito, T., Owen, D. M., Jiang, F., Marcotrigiano, J. & Gale, M. Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature* **454**, 523–527 (2008).

51. Lhomme, S. et al. Hepatitis E virus: how it escapes host innate immunity. *Vaccines* **8**, 422 (2020).
52. Sayed, I. M. et al. Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver. *Gut* **66**, 920–929 (2017).
53. Yu, C. et al. Pathogenesis of hepatitis E virus and hepatitis C virus in chimpanzees: similarities and differences. *J. Virol.* **84**, 11264–11278 (2010).
54. Moal, V. et al. Chronic hepatitis E virus infection is specifically associated with an interferon-related transcriptional program. *J. Infect. Dis.* **207**, 125–132 (2013).
55. Wu, X. et al. Pan-genotype hepatitis E virus replication in stem cell-derived hepatocellular systems. *Gastroenterology* **154**, 663–674.e7 (2018).
56. Marion, O. et al. Hepatitis E virus replication in human intestinal cells. *Gut* **69**, 901–910 (2020).
57. Murata, K. et al. IFN-λ3 as a host immune response in acute hepatitis E virus infection. *Cytokine* **125**, 154816 (2020).
58. Gouilly, J. et al. Genotype specific pathogenicity of hepatitis E virus at the human maternal-fetal interface. *Nat. Commun.* **9**, 4748 (2018).
59. Purcell, R. H. et al. Pathobiology of hepatitis E: lessons learned from primate models. *Emerg. Microbes Infect.* **2**, e9 (2013).
60. Allweiss, L. et al. Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation. *J. Hepatol.* **64**, 1033–1040 (2016).
61. van de Garde, M. D. B. et al. Interferon-alpha treatment rapidly clears Hepatitis E virus infection in humanized mice. *Sci. Rep.* **7**, 8267 (2017).
62. Knegendorf, L. et al. Hepatitis E virus replication and interferon responses in human placental cells. *Hepatol. Commun.* **2**, 173–187 (2018).
63. Moal, V. et al. Hepatitis E virus serological testing in kidney transplant recipients with elevated liver enzymes in 2007-2011 in southeastern France. *Diagn. Microbiol. Infect. Dis.* **76**, 116–118 (2013).
64. Schoggins, J. W. & Rice, C. M. Interferon-stimulated genes and their antiviral effector functions. *Curr. Opin. Virol.* **1**, 519–525 (2011).
65. Schoggins, J. W. et al. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* **472**, 481–485 (2011).
66. Zhang, F. et al. Hepatitis E genotype 4 virus from feces of monkeys infected experimentally can be cultured in PLC/PRF/5 cells and upregulate host interferon-inducible genes. *J. Med. Virol.* **86**, 1736–1744 (2014).
67. Sooryanarain, H. et al. ISG15 modulates type I interferon signaling and the antiviral response during hepatitis E virus replication. *J. Virol.* **91**, e00621-17 (2017).
68. Xu, L. et al. IFN regulatory factor 1 restricts hepatitis E virus replication by activating STAT1 to induce antiviral IFN-stimulated genes. *FASEB J.* **30**, 3352–3367 (2016).
69. Glitscher, M. et al. Identification of the interferon-inducible GTPase GBP1 as major restriction factor for the hepatitis E virus. *J. Virol.* **95**, e01564-20 (2021).
70. Wang, M., Huang, Y., He, M., Peng, W.-J. & Tian, D.-Y. Effects of hepatitis E virus infection on interferon production via ISG15. *World J. Gastroenterol.* **24**, 2173–2180 (2018).
71. Hermann, M. & Bogunovic, D. ISG15: in sickness and in health. *Trends Immunol.* **38**, 79–93 (2017).
72. Daugherty, M. D. & Malik, H. S. Rules of engagement: molecular insights from host-virus arms races. *Annu. Rev. Genet.* **46**, 677–700 (2012).
73. Hu, B., Huang, S. & Yin, L. The cytokine storm and COVID-19. *J. Med. Virol.* **93**, 250–256 (2021).
74. Li, H., Huang, M.-H., Jiang, J.-D. & Peng, Z.-G. Hepatitis C: from inflammatory pathogenesis to anti-inflammatory/hepatoprotective therapy. *World J. Gastroenterol.* **24**, 5297–5311 (2018).
75. Li, Y. et al. Hepatitis E virus infection activates NOD-like receptor family pyrin domain-containing 3 inflammasome antagonizing interferon response but therapeutically targetable. *Hepatology* **75**, 196–212 (2022).
76. Debes, J. D., Groothuismink, Z. M. A., Doukas, M., de Man, R. A. & Boonstra, A. Immune dissociation during acute hepatitis E infection. *Int. J. Infect. Dis.* **87**, 39–42 (2019).
77. Saravanabalaji, S. et al. Viral load, antibody titers and recombinant open reading frame 2 protein-induced TH1/TH2 cytokines and cellular immune responses in self-limiting and fulminant hepatitis E. *Intervirology* **52**, 78–85 (2009).
78. Kumar, A. et al. Association of cytokines in hepatitis E with pregnancy outcome. *Cytokine* **65**, 95–104 (2014).
79. Behrendt, P. et al. Exacerbation of hepatitis E virus infection during anti-TNFα treatment. *Joint Bone Spine* **84**, 217–219 (2017).
80. Kinast, V. et al. Janus kinase-inhibition modulates hepatitis E virus infection. *Antivir. Res.* **217**, 105690 (2023).
81. Mishra, N. & Arankalle, V. A. Association of polymorphisms in the promoter regions of TNF-α (-308) with susceptibility to hepatitis E virus and TNF-α (-1031) and IFN-γ (+874) genes with clinical outcome of hepatitis E infection in India. *J. Hepatol.* **55**, 1227–1234 (2011).
82. Devi, S. G., Kumar, A., Kar, P., Husain, S. A. & Sharma, S. Association of pregnancy outcome with cytokine gene polymorphisms in HEV infection during pregnancy. *J. Med. Virol.* **86**, 1366–1376 (2014).
83. Sung, P. S. et al. Roles of unphosphorylated ISGF3 in HCV infection and interferon responsiveness. *Proc. Natl Acad. Sci. USA* **112**, 10443–10448 (2015).
84. Shin, E.-C., Sung, P. S. & Park, S.-H. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat. Rev. Immunol.* **16**, 509–523 (2016).
85. Todt, D. et al. Antiviral activities of different interferon types and subtypes against hepatitis E virus replication. *Antimicrob. Agents Chemother.* **60**, 2132–2139 (2016).
86. Zhou, X. et al. Disparity of basal and therapeutically activated interferon signalling in constraining hepatitis E virus infection. *J. Viral Hepat.* **23**, 294–304 (2016).
87. Kamar, N. et al. Pegylated interferon-α for treating chronic hepatitis E virus infection after liver transplantation. *Clin. Infect. Dis.* **50**, e30–e33 (2010).
88. Haagsma, E. B., Riezebos-Brilman, A., van den Berg, A. P., Porte, R. J. & Niesters, H. G. M. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transplant.* **16**, 474–477 (2010).
89. Sarasin-Filipowicz, M. et al. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc. Natl Acad. Sci. USA* **105**, 7034–7039 (2008).
90. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C: final update of the series. *J. Hepatol.* **73**, 1170–1218 (2020).
91. Enomoto, N. et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. *J. Clin. Invest.* **96**, 224–230 (1995).
92. World Health Organization. *Hepatitis C* https://www.who.int/news-room/fact-sheets/detail/hepatitis-c (2024).
93. Ge, D. et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* **461**, 399–401 (2009).
94. Prokunina-Olsson, L. et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat. Genet.* **45**, 164–171 (2013).
95. Thomas, D. L. et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* **461**, 798–801 (2009).
96. Kamar, N. et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology* **140**, 1481–1489 (2011).
97. Plosker, G. L. & Foster, R. H. Tacrolimus: a further update of its pharmacology and therapeutic use in the management of organ transplantation. *Drugs* **59**, 323–389 (2000).
98. Mejia, J. C., Basu, A. & Shapiro, R. In: *Kidney Transplantation — Principles and Practice* (eds Morris, P. J. & Knechtle, S. J.) 231–249 (Elsevier, 2014).
99. Wang, Y. et al. Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. *Gastroenterology* **146**, 1775–1783 (2014).
100. Wang, Y., Metselaar, H. J., Peppelenbosch, M. P. & Pan, Q. Chronic hepatitis E in solid-organ transplantation: the key implications of immunosuppressants. *Curr. Opin. Infect. Dis.* **27**, 303–308 (2014).
101. Nan, Y. et al. Hepatitis E virus inhibits type I interferon induction by ORF1 products. *J. Virol.* **88**, 11924–11932 (2014).
102. Bagdassarian, E. et al. The amino-terminal region of hepatitis E virus ORF1 containing a methyltransferase (Met) and a papain-like cysteine protease (PCP) domain counteracts type I interferon response. *Viruses* **10**, 726 (2018).
103. Kim, E. & Myoung, J. Hepatitis E virus papain-like cysteine protease inhibits type I interferon induction by down-regulating melanoma differentiation-associated gene 5. *J. Microbiol. Biotechnol.* **28**, 1908–1915 (2018).
104. Kang, S. et al. Hepatitis E virus methyltransferase inhibits type I interferon induction by targeting RIG-I. *J. Microbiol. Biotechnol.* **28**, 1554–1562 (2018).
105. Myoung, J., Lee, J. Y. & Min, K. S. Methyltransferase of a cell culture-adapted hepatitis E inhibits the MDA5 receptor signaling pathway. *J. Microbiol.* **57**, 1126–1131 (2019).
106. Myoung, J. & Min, K. Dose-dependent inhibition of melanoma differentiation-associated gene 5-mediated activation of type I interferon responses by methyltransferase of hepatitis E virus. *J. Microbiol. Biotechnol.* **29**, 1137–1143 (2019).
107. Dong, C. et al. Suppression of interferon-α signaling by hepatitis E virus. *Hepatology* **55**, 1324–1332 (2012).
108. He, M. et al. The ORF3 protein of genotype 1 hepatitis E virus suppresses TLR3-induced NF-κB signaling via TRADD and RIP1. *Sci. Rep.* **6**, 27597 (2016).
109. Lei, Q. et al. HEV ORF3 downregulates TLR7 to inhibit the generation of type I interferon via impairment of multiple signaling pathways. *Sci. Rep.* **8**, 8585 (2018).
110. Lei, Q. et al. ORF3 of hepatitis E virus inhibits the expression of proinflammatory cytokines and chemotactic factors in LPS-stimulated human PMA-THP1 cells by inhibiting NF-κB pathway. *Viral Immunol.* **29**, 105–111 (2016).
111. Sayed, I. M. et al. Replication of hepatitis E virus (HEV) in primary human-derived monocytes and macrophages in vitro. *Vaccines* **8**, 239 (2020).
112. Surjit, M., Varshney, B. & Lal, S. K. The ORF2 glycoprotein of hepatitis E virus inhibits cellular NF-κB activity by blocking ubiquitination mediated proteasomal degradation of IκBα in human hepatoma cells. *BMC Biochem.* **13**, 7 (2012).
113. Tian, Y. et al. Systematic identification of hepatitis E virus ORF2 interactome reveals that TMEM134 engages in ORF2-mediated NF-κB pathway. *Virus Res.* **228**, 102–108 (2017).
114. Lin, S. et al. The capsid protein of hepatitis E virus inhibits interferon induction via its N-terminal arginine-rich motif. *Viruses* **11**, 1050 (2019).
115. Hingane, S., Joshi, N., Surjit, M. & Ranjith-Kumar, C. T. Hepatitis E virus ORF2 inhibits RIG-I mediated interferon response. *Front. Microbiol.* **11**, 656 (2020).
116. Meister, T. L., Bruening, J., Todt, D. & Steinmann, E. Cell culture systems for the study of hepatitis E virus. *Antivir. Res.* **163**, 34–49 (2019).
117. LeDesma, R. et al. Structural features stabilized by divalent cation coordination within hepatitis E virus ORF1 are critical for viral replication. *eLife* **12**, e80529 (2023).
118. Ju, C. & Tacke, F. Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. *Cell. Mol. Immunol.* **13**, 316–327 (2016).
119. Martinon, F., Mayor, A. & Tschopp, J. The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* **27**, 229–265 (2009).
120. Björkström, N. K., Strunz, B. & Ljunggren, H.-G. Natural killer cells in antiviral immunity. *Nat. Rev. Immunol.* **22**, 112–123 (2022).
121. Caligiuri, M. A. Human natural killer cells. *Blood* **112**, 461–469 (2008).

122. Rehermann, B. Natural killer cells in viral hepatitis. *Cell. Mol. Gastroenterol. Hepatol.* **1**, 578–588 (2015).
123. Srivastava, R., Aggarwal, R., Bhagat, M. R., Chowdhury, A. & Naik, S. Alterations in natural killer cells and natural killer T cells during acute viral hepatitis E. *J. Viral Hepat.* **15**, 910–916 (2008).
124. Das, R. & Tripathy, A. Increased expressions of NKp44, NKp46 on NK/NKT-like cells are associated with impaired cytolytic function in self-limiting hepatitis E infection. *Med. Microbiol. Immunol.* **203**, 303–314 (2014).
125. Prabhu, S. B. et al. Study of cellular immune response against Hepatitis E virus (HEV). *J. Viral Hepat.* **18**, 587–594 (2011).
126. Barragué, H. et al. Mobilization of γδ T cells and IL-10 production at the acute phase of hepatitis E virus infection in cytomegalovirus carriers. *J. Immunol.* **206**, 1027–1038 (2021).
127. Vantourout, P. & Hayday, A. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat. Rev. Immunol.* **13**, 88–100 (2013).
128. Lunemann, S. et al. Compromised function of natural killer cells in acute and chronic viral hepatitis. *J. Infect. Dis.* **209**, 1362–1373 (2014).
129. Khakoo, S. I. et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* **305**, 872–874 (2004).
130. Alter, G. et al. HIV-1 adaptation to NK-cell-mediated immune pressure. *Nature* **476**, 96–100 (2011).
131. Klenerman, P. & Thimme, R. T cell responses in hepatitis C: the good, the bad and the unconventional. *Gut* **61**, 1226–1234 (2012).
132. Hoogeveen, R. C. et al. Hepatitis B virus-specific CD4 T cell responses differentiate functional cure from chronic surface antigen$^+$ infection. *J. Hepatol.* **77**, 1276–1286 (2022).
133. Kumar, B. V., Connors, T. J. & Farber, D. L. Human T cell development, localization, and function throughout life. *Immunity* **48**, 202–213 (2018).
134. Srivastava, R. et al. Cellular immune responses in acute hepatitis E virus infection to the viral open reading frame 2 protein. *Viral Immunol.* **20**, 56–65 (2007).
135. Brown, A. et al. Characterization of the specificity, functionality, and durability of host T-cell responses against the full-length hepatitis E virus. *Hepatology* **64**, 1934–1950 (2016).
136. Suneetha, P. V. et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. *Hepatology* **55**, 695–708 (2012).
First association between chronic hepatitis E and impaired HEV-specific T cell responses and restoration of HEV-specific T cell responses through blockage of the PD1 or CTLA4 pathways.
137. Agrawal, V., Goel, A., Rawat, A., Naik, S. & Aggarwal, R. Histological and immunohistochemical features in fatal acute fulminant hepatitis E. *Indian J. Pathol. Microbiol.* **55**, 22–27 (2012).
138. Husain, M. M., Aggarwal, R., Kumar, D., Jameel, S. & Naik, S. Effector T cells immune reactivity among patients with acute hepatitis E. *J. Viral Hepat.* **18**, e603–e608 (2011).
139. Tripathy, A. S., Das, R., Rathod, S. B. & Arankalle, V. A. Cytokine profiles, CTL response and T cell frequencies in the peripheral blood of acute patients and individuals recovered from hepatitis E infection. *PLoS One* **7**, e31822 (2012).
140. Gisa, A. et al. Cross-genotype-specific T-cell responses in acute hepatitis E virus (HEV) infection. *J. Viral Hepat.* **23**, 305–315 (2016).
This study described the discovery of cross-genotype T cell responses that might suggest a potential role in cross-genotype-specific protection between HEV genotypes 1 and 3.
141. El Costa, H. et al. Effector memory CD8 T cell response elicits hepatitis E virus genotype 3 pathogenesis in the elderly. *PLoS Pathog.* **17**, e1009367 (2021).
142. Shata, M. T. et al. Characterization of hepatitis E-specific cell-mediated immune response using IFN-γ ELISPOT assay. *J. Immunol. Methods* **328**, 152–161 (2007).
143. Wu, T. et al. Specific cellular immune response in hepatitis E patients. *Intervirology* **51**, 322–327 (2008).
144. Al-Ayoubi, J. et al. Hepatitis E virus ORF 1 induces proliferative and functional T-cell responses in patients with ongoing and resolved hepatitis E. *Liver Int.* **38**, 266–277 (2018).
145. Kemming, J. et al. Mechanisms of CD8$^+$ T-cell failure in chronic hepatitis E virus infection. *J. Hepatol.* **77**, 978–990 (2022).
First in-depth analysis of T cell responses during chronic HEV3 infection; observation of HEV-specific CD8$^+$ T cell responses that displayed features of exhaustion and identification of viral escape variant of a T cell epitope.
146. Aggarwal, R. et al. T-cell epitope mapping of ORF2 and ORF3 proteins of human hepatitis E virus. *J. Viral Hepat.* **14**, 283–292 (2007).
147. TrehanPati, N. et al. Gene expression profiles of T cells from hepatitis E virus infected patients in acute and resolving phase. *J. Clin. Immunol.* **31**, 498–508 (2011).
148. Dalton, H. R. et al. Autochthonous hepatitis E in southwest England. *J. Viral Hepat.* **14**, 304–309 (2007).
149. Kamar, N., Dalton, H. R., Abravanel, F. & Izopet, J. Hepatitis E virus infection. *Clin. Microbiol. Rev.* **27**, 116–138 (2014).
150. Manka, P. et al. Hepatitis E virus infection as a possible cause of acute liver failure in Europe. *Clin. Gastroenterol. Hepatol.* **13**, 1836–1842.e2 (2015).
151. Pal, R. et al. Immunological alterations in pregnant women with acute hepatitis E. *J. Gastroenterol. Hepatol.* **20**, 1094–1101 (2005).
152. Wu, J. et al. Th1/Th2 cells and associated cytokines in acute hepatitis E and related acute liver failure. *J. Immunol. Res.* **2020**, 6027361 (2020).
153. Montpellier, C. et al. Hepatitis E virus lifecycle and identification of 3 forms of the ORF2 capsid protein. *Gastroenterology* **154**, 211–223.e8 (2018).
154. Gumber, D. & Wang, L. D. Improving CAR-T immunotherapy: overcoming the challenges of T cell exhaustion. *eBioMedicine* **77**, 103941 (2022).
155. Khan, O. et al. TOX transcriptionally and epigenetically programs CD8$^+$ T cell exhaustion. *Nature* **571**, 211–218 (2019).
156. Scott, A. C. et al. TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* **571**, 270–274 (2019).
157. Alfei, F. et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* **571**, 265–269 (2019).
158. Chen, J. et al. NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* **567**, 530–534 (2019).
159. Uhl, M. et al. SD-208, a novel transforming growth factor β receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells in vitro and in vivo. *Cancer Res.* **64**, 7954–7961 (2004).
160. Kumar, J. et al. Deletion of Cbl-b inhibits CD8$^+$ T-cell exhaustion and promotes CAR T-cell function. *J. Immunother. Cancer* **9**, e001688 (2021).
161. Ford, B. R. et al. Tumor microenvironmental signals reshape chromatin landscapes to limit the functional potential of exhausted T cells. *Sci. Immunol.* **7**, eabj9123 (2022).
162. Soon, C. F. et al. Defining virus-specific CD8$^+$ TCR repertoires for therapeutic regeneration of T cells against chronic hepatitis E. *J. Hepatol.* **71**, 673–684 (2019).
Identification of T cell receptors targeting HLA-A2 restricted HEV-specific CD8$^+$ T cell epitopes, which can function as a redirected TCR therapy approach for patients with chronic HEV.
163. Soon, C. F. et al. Hepatitis E virus (HEV)-specific T cell receptor cross-recognition: implications for immunotherapy. *Front. Immunol.* **10**, 2076 (2019).
164. Bertoletti, A. et al. Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. *Nature* **369**, 407–410 (1994).
165. McMichael, A. T cell responses and viral escape. *Cell* **93**, 673–676 (1998).
166. Thimme, R. T cell immunity to hepatitis C virus: lessons for a prophylactic vaccine. *J. Hepatol.* **74**, 220–229 (2021).
167. Katayama, Y., Yokota, R., Akiyama, T. & Kobayashi, T. J. Machine learning approaches to TCR repertoire analysis. *Front. Immunol.* **13**, 858057 (2022).
168. Lu, T. et al. Deep learning-based prediction of the T cell receptor–antigen binding specificity. *Nat. Mach. Intell.* **3**, 864–875 (2021).
169. Shoukat, M. S. et al. Use of machine learning to identify a T cell response to SARS-CoV-2. *Cell Rep. Med.* **2**, 100192 (2021).
170. Kulkarni, S. P., Sharma, M. & Tripathy, A. S. Antibody and memory B cell responses in hepatitis E recovered individuals, 1-30 years post hepatitis E virus infection. *Sci. Rep.* **9**, 4090 (2019).
171. Srivastava, R. et al. Adaptive immune responses during acute uncomplicated and fulminant hepatitis E. *J. Gastroenterol. Hepatol.* **26**, 306–311 (2011).
172. Sharma, M. & Tripathy, A. S. Regulatory role of B cells and its subsets in hepatitis E virus infection. *BioMed. Res. Int.* **2022**, 7932150 (2022).
173. Chau, K. H. et al. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. *J. Med. Virol.* **40**, 334–338 (1993).
174. Clayson, E. T. et al. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J. Infect. Dis.* **172**, 927–933 (1995).
175. Huang, S. et al. Profile of acute infectious markers in sporadic hepatitis E. *PLoS One* **5**, e13560 (2010).
176. Bryan, J. P. et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. *J. Infect. Dis.* **170**, 517–521 (1994).
177. Chandra, N. S., Sharma, A., Malhotra, B. & Rai, R. R. Dynamics of HEV viremia, fecal shedding and its relationship with transaminases and antibody response in patients with sporadic acute hepatitis E. *Virol. J.* **7**, 213 (2010).
178. Zhang, J. Z. et al. Occurrence of hepatitis E virus IgM, low avidity IgG serum antibodies, and viremia in sporadic cases of non-A, -B, and -C acute hepatitis. *J. Med. Virol.* **66**, 40–48 (2002).
179. Bendall, R., Ellis, V., Ijaz, S., Thurairajah, P. & Dalton, H. R. Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response. *J. Med. Virol.* **80**, 95–101 (2008).
180. Schemmerer, M., Rauh, C., Jilg, W. & Wenzel, J. J. Time course of hepatitis E-specific antibodies in adults. *J. Viral Hepat.* **24**, 75–79 (2017).
181. Sultankhuroo, M. Hepatitis E and long-term antibody status. *Lancet* **341**, 1355 (1993).
182. Takahashi, M. et al. Hepatitis E virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: characterization of HEV virions in blood circulation. *J. Clin. Microbiol.* **48**, 1112–1125 (2010).
183. Vollmer, T., Diekmann, J., Eberhardt, M., Knabbe, C. & Dreier, J. Hepatitis E in blood donors: investigation of the natural course of asymptomatic infection, Germany, 2011. *Euro Surveill.* **21**, 30332 (2016).
184. Kraef, C. et al. Course of HEV viremia and anti-HEV IgM/IgG response in asymptomatic blood donors. *J. Clin. Virol.* **105**, 26–30 (2018).
185. Legrand-Abravanel, F. et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J. Infect. Dis.* **202**, 835–844 (2010).
186. Choi, M. et al. Prevalence and clinical correlates of chronic hepatitis E infection in German renal transplant recipients with elevated liver enzymes. *Transplant. Direct* **4**, e341 (2018).
187. Haagsma, E. B. et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transplant.* **14**, 547–553 (2008).

188. Pas, S. D. et al. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerg. Infect. Dis.* **18**, 869–872 (2012).
189. Tamura, A. et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. *Hepatol. Res.* **37**, 113–120 (2007).
190. Pischke, S. et al. Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. *Liver Transplant.* **16**, 74–82 (2010).
191. Rentenaar, R. J. et al. Immune responsiveness in renal transplant recipients: mycophenolic acid severely depresses humoral immunity in vivo. *Kidney Int.* **62**, 319–328 (2002).
192. Luo, H. et al. Inhibition of in vitro immunoglobulin production by rapamycin. *Transplantation* **53**, 1071–1076 (1992).
193. Aagaard-Tillery, K. M. & Jelinek, D. F. Inhibition of human B lymphocyte cell cycle progression and differentiation by rapamycin. *Cell. Immunol.* **156**, 493–507 (1994).
194. Gardinali, N. R. et al. Cynomolgus monkeys are successfully and persistently infected with hepatitis E virus genotype 3 (HEV-3) after long-term immunosuppressive therapy. *PLoS One* **12**, e0174070 (2017).
195. Tsarev, S. A. et al. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc. Natl Acad. Sci. USA* **91**, 10198–10202 (1994).
196. Khuroo, M. S. & Khuroo, M. S. Seroepidemiology of a second epidemic of hepatitis E in a population that had recorded first epidemic 30 years before and has been under surveillance since then. *Hepatol. Int.* **4**, 494–499 (2010).
197. Servant-Delmas, A. et al. New insights into the natural history of hepatitis E virus infection through a longitudinal study of multitransfused immunocompetent patients in France. *J. Viral Hepat.* **23**, 569–575 (2016).
198. Faber, M. et al. Hepatitis E virus seroprevalence, seroincidence and seroreversion in the German adult population. *J. Viral Hepat.* **25**, 752–758 (2018).
199. Hogema, B. M., Molier, M., Slot, E. & Zaaijer, H. L. Past and present of hepatitis E in the Netherlands. *Transfusion* **54**, 3092–3096 (2014).
200. Choi, Y., Zhang, X. & Skinner, B. Analysis of IgG anti-HEV antibody protective levels during hepatitis E virus reinfection in experimentally infected rhesus macaques. *J. Infect. Dis.* **219**, 916–924 (2019).
201. Arankalle, V. A., Chadha, M. S. & Chobe, L. P. Long-term serological follow up and cross-challenge studies in rhesus monkeys experimentally infected with hepatitis E virus. *J. Hepatol.* **30**, 199–204 (1999).
202. Huang, W. et al. Cross-protection of hepatitis E virus genotypes 1 and 4 in rhesus macaques. *J. Med. Virol.* **80**, 824–832 (2008).
203. Arankalle, V. A., Chadha, M. S., Chobe, L. P., Nair, R. & Banerjee, K. Cross-challenge studies in rhesus monkeys employing different Indian isolates of hepatitis E virus. *J. Med. Virol.* **46**, 358–363 (1995).
204. Abravanel, F. et al. Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. *J. Infect. Dis.* **209**, 1900–1906 (2014).
205. Ferguson, M., Walker, D., Mast, E. & Fields, H. Report of a collaborative study to assess the suitability of a reference reagent for antibodies to hepatitis E virus. *Biologicals* **30**, 43–48 (2002).
The authors identified the WHO HEV IgG reference reagent 95/584 and found it to be suitable to serve as a reference reagent for hepatitis E serum IgG and established the reagent as an interim reference reagent for human anti-hepatitis E serum.
206. Behrendt, P. & Wedemeyer, H. Impfstoffe gegen hepatitis E: Wo stehen wir? *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* **65**, 192–201 (2022).
207. Zhang, J. et al. Long-term efficacy of a hepatitis E vaccine. *N. Engl. J. Med.* **372**, 914–922 (2015).
208. Zhu, F.-C. et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet* **376**, 895–902 (2010).
First clinical trial examining a truncated ORF2 termed HEV 239 (trade name Hecolin) for the prevention and vaccination of HEV1 infection.
209. World Health Organization. *Safety of Hepatitis E Vaccines* https://www.who.int/groups/global-advisory-committee-on-vaccine-safety/topics/hepatitis-e-vaccines (2023).
210. Li, S. W. et al. A bacterially expressed particulate hepatitis E vaccine: antigenicity, immunogenicity and protectivity on primates. *Vaccine* **23**, 2893–2901 (2005).
211. Ciglenecki, I. et al. The first reactive vaccination campaign against hepatitis E. *Lancet Infect. Dis.* **22**, 1110–1111 (2022).
212. Nesbitt, R. C. et al. Vaccination coverage and adverse events following a reactive vaccination campaign against hepatitis E in Bentiu displaced persons camp, South Sudan. *PLoS Negl. Trop. Dis.* **18**, e0011661 (2024).
213. Purcell, R. H. et al. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine* **21**, 2607–2615 (2003).
214. Safary, A. Perspectives of vaccination against hepatitis E. *Intervirology* **44**, 162–166 (2001).
215. Emerson, S. U. & Purcell, R. H. Recombinant vaccines for hepatitis E. *Trends Mol. Med.* **7**, 462–466 (2001).
216. Shrestha, M. P. et al. Safety and efficacy of a recombinant hepatitis E vaccine. *N. Engl. J. Med.* **356**, 895–903 (2007).
217. Stevenson, P. Nepal calls the shots in hepatitis E virus vaccine trial. *Lancet* **355**, 1623 (2000).
218. Basu, S. & Lurie, P. Hepatitis E vaccine. *N. Engl. J. Med.* **356**, 2421–2422 (2007).
219. Andrews, J. U.S. Military sponsored vaccine trials and la resistance in Nepal. *Am. J. Bioeth.* **5**, W1–W3 (2005).
220. Meng, J. et al. Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. *Virology* **288**, 203–211 (2001).
221. Wen, J. et al. Immunogenicity difference between two hepatitis E vaccines derived from genotype 1 and 4. *Antivir. Res.* **128**, 36–42 (2016).
222. Cao, Y.-F. et al. A phase 1 randomized open-label clinical study to evaluate the safety and tolerability of a novel recombinant hepatitis E vaccine. *Vaccine* **35**, 5073–5080 (2017).
223. Lynch, J. A. et al. Hepatitis E vaccine — illuminating the barriers to use. *PLoS Negl. Trop. Dis.* **17**, e0010969 (2023).
224. World Health Organization. *Who Position Paper on Hepatitis E Vaccines — May 2015* https://www.who.int/publications/i/item/WER9018-185-200 (2015).
225. Reyes, G. R. et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* **247**, 1335–1339 (1990).
Isolation and cloning of the cDNA HEV genome.
226. Tam, A. W. et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. *Virology* **185**, 120–131 (1991).
227. Panda, S. K., Ansari, I. H., Durgapal, H., Agrawal, S. & Jameel, S. The in vitro-synthesized RNA from a cDNA clone of hepatitis E virus is infectious. *J. Virol.* **74**, 2430–2437 (2000).
228. Emerson, S. U. et al. Recombinant hepatitis E virus genomes infectious for primates: importance of capping and discovery of a cis-reactive element. *Proc. Natl Acad. Sci. USA* **98**, 15270–15275 (2001).
229. Tei, S., Kitajima, N., Takahashi, K. & Mishiro, S. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* **362**, 371–373 (2003).
First direct evidence of zoonotic transmission of hepatitis E virus.
230. Baylis, S. A. et al. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. *Emerg. Infect. Dis.* **19**, 729–735 (2013).
231. Kamar, N. et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N. Engl. J. Med.* **370**, 1111–1120 (2014).
Ribavirin as monotherapy for the treatment of chronic HEV infection.
232. Xing, L. et al. Structure of hepatitis E virion-sized particle reveals an RNA-dependent viral assembly pathway. *J. Biol. Chem.* **285**, 33175–33183 (2010).
Identification of virion-sized capsids with a *T*=3 icosahedral lattice structure by cryo-electron microscopy; the authors also demonstrate that RNA binding is the extrinsic factor essential for the assembly of HEV native capsids.
233. Ralfs, P. et al. Soluble ORF2 protein enhances HEV replication and induces long-lasting antibody response and protective immunity in vivo. *Hepatology* **78**, 1867–1881 (2023).
234. Yin, X. et al. Origin, antigenicity, and function of a secreted form of ORF2 in hepatitis E virus infection. *Proc. Natl Acad. Sci. USA* **115**, 4773–4778 (2018).
235. Meister, T. L. et al. A ribavirin-induced ORF2 single-nucleotide variant produces defective hepatitis E virus particles with immune decoy function. *Proc. Natl Acad. Sci. USA* **119**, e2202653119 (2022).
236. Yamashita, T. et al. Biological and immunological characteristics of hepatitis E virus-like particles based on the crystal structure. *Proc. Natl Acad. Sci. USA* **106**, 12986–12991 (2009).
237. Guu, T. S. Y. et al. Structure of the hepatitis E virus-like particle suggests mechanisms for virus assembly and receptor binding. *Proc. Natl Acad. Sci. USA* **106**, 12992–12997 (2009).
238. Schofield, D. J., Glamann, J., Emerson, S. U. & Purcell, R. H. Identification by phage display and characterization of two neutralizing chimpanzee monoclonal antibodies to the hepatitis E virus capsid protein. *J. Virol.* **74**, 5548–5555 (2000).
239. Zheng, Q. et al. Viral neutralization by antibody-imposed physical disruption. *Proc. Natl Acad. Sci. USA* **116**, 26933–26940 (2019).
240. Li, S. et al. Dimerization of hepatitis E virus capsid protein E2s domain is essential for virus-host interaction. *PLoS Pathog.* **5**, e1000537 (2009).
241. Xing, L. et al. Spatial configuration of hepatitis E virus antigenic domain. *J. Virol.* **85**, 1117–1124 (2011).
242. Zhang, J. et al. Analysis of hepatitis E virus neutralization sites using monoclonal antibodies directed against a virus capsid protein. *Vaccine* **23**, 2881–2892 (2005).
243. Xing, L. et al. Recombinant hepatitis E capsid protein self-assembles into a dual-domain T = 1 particle presenting native virus epitopes. *Virology* **265**, 35–45 (1999).
244. Li, T., Takeda, N. & Miyamura, T. Oral administration of hepatitis E virus-like particles induces a systemic and mucosal immune response in mice. *Vaccine* **19**, 3476–3484 (2001).
245. Li, T. C. et al. Expression and self-assembly of empty virus-like particles of hepatitis E virus. *J. Virol.* **71**, 7207–7213 (1997).
246. Shima, R. et al. Production of hepatitis E virus-like particles presenting multiple foreign epitopes by co-infection of recombinant baculoviruses. *Sci. Rep.* **6**, 21638 (2016).
247. Schofield, D. J., Purcell, R. H., Nguyen, H. T. & Emerson, S. U. Monoclonal antibodies that neutralize HEV recognize an antigenic site at the carboxyterminus of an ORF2 protein vaccine. *Vaccine* **22**, 257–267 (2003).
248. Li, T.-C. et al. Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus. *J. Virol.* **79**, 12999–13006 (2005).
249. Purdy, M. A. et al. Preliminary evidence that a trpE-HEV fusion protein protects cynomolgus macaques against challenge with wild-type hepatitis E virus (HEV). *J. Med. Virol.* **41**, 90–94 (1993).
250. Ghabrah, T. M. et al. Comparison of tests for antibody to hepatitis E virus. *J. Med. Virol.* **55**, 134–137 (1998).
251. Kaur, M. et al. Human linear B-cell epitopes encoded by the hepatitis E virus include determinants in the RNA-dependent RNA polymerase. *Proc. Natl Acad. Sci. USA* **89**, 3855–3858 (1992).
252. Khudyakov, Y., Favorov, M. O., Jue, D. L., Hine, T. K. & Fields, H. A. Immunodominant antigenic regions in a structural protein of the hepatitis E virus. *Virology* **198**, 390–393 (1994).

253. Riddell, M. A., Li, F. & Anderson, D. A. Identification of immunodominant and conformational epitopes in the capsid protein of hepatitis E virus by using monoclonal antibodies. *J. Virol.* **74**, 8011–8017 (2000).
254. Tang, X. et al. Structural basis for the neutralization and genotype specificity of hepatitis E virus. *Proc. Natl Acad. Sci. USA* **108**, 10266–10271 (2011).
255. Emerson, S. U. et al. Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. *J. Gen. Virol.* **87**, 697–704 (2006).
256. Gu, Y. et al. Structural basis for the neutralization of hepatitis E virus by a cross-genotype antibody. *Cell Res.* **25**, 604–620 (2015).
257. Li, S.-W. et al. Mutational analysis of essential interactions involved in the assembly of hepatitis E virus capsid. *J. Biol. Chem.* **280**, 3400–3406 (2005).

Acknowledgements
The authors thank Jörg Timm, Volker Kinast and Viet Loan Dao Thi for comments on the manuscript. Moreover, we thank all members of the Department for Molecular and Medical Virology, Ruhr University Bochum, for helpful support, suggestions and discussions. E.S. was supported by grants from the German Research Foundation (DFG, grant number: 510558817) and the medical faculty of the Ruhr University Bochum (InnovationsFoRUM, grant number: IF-018N-22). H.W. and E.S. were funded by a grant from the German Centre for Infection Research (DZIF). Y.B. was supported by the medical faculty of the Ruhr University Bochum (FoRUM, grant number: F1030-2021).

Author contributions
Y.B., M.K., H.W. and E.S. developed the concept and coordinated the manuscript. Y.B. and M.K. wrote the manuscript and generated the original version of the figures. All authors reviewed the final manuscript.

Competing interests
H.W. consults for, is on the speakers' bureau for, received grants from, and has other interests in Gilead Sciences, Inc. He consults for, received grants from, and has other interests in Falk. He consults for and has other interests in Merck Sharp & Dohme. He consults for and advises Roche. He consults for and is on the speakers' bureau for Pfizer Inc. He is on the speakers' bureau for the Falk Foundation. The other authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41575-024-00950-z.

Peer review information *Nature Reviews Gastroenterology & Hepatology* thanks Jacques Izopet; Alexander Ploss; and Jun Zhang, who co-reviewed with Zizheng Zheng, for their contribution to the peer review of this work.