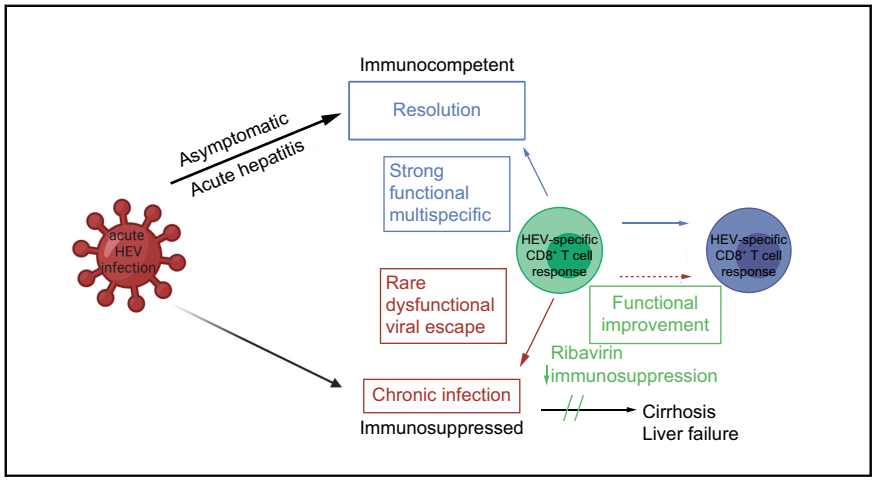
Research Article
Viral Hepatitis

JOURNAL
OF HEPATOLOGY

Mechanisms of CD8+ T-cell failure in chronic hepatitis E virus infection

Graphical abstract



Highlights

- Persistent HEV genotype 3 infection occurs in about 50-70% of immunosuppressed patients.
- HEV-specific CD8+ T cell responses in self-limiting HEV infection were vigorous and formed a functional memory.
- Chronic HEV infection was associated with diminished HEV-specific CD8+ T cell responses that displayed features of exhaustion.
- In a minority, CD8+ T-cell driven viral escape contributed to the failure of the HEV-specific CD8+ T-cell response.

Authors

Janine Kemming, Swantje Gundlach, Marcus Panning, …, Maike Hofmann, Nicole Fischer,
Christoph Neumann-Haefelin

Correspondence

christoph.neumann-haefelin@uniklinik-freiburg.de
(C. Neumann-Haefelin).

Lay summary

Hepatitis E virus (HEV) infection is usually cleared spontaneously (without treatment) in patients with fully functioning immune systems. In immunosuppressed patients, chronic HEV infection is common and can progress rapidly to cirrhosis and liver failure. Herein, we identified the presence of HEV-specific CD8+ T cells (a specific type of immune cell that can target HEV) in immunosuppressed patients, but we show that these cells do not function properly. This dysfunction appears to play a role in the development of chronic HEV infection in vulnerable patients.

https://doi.org/10.1016/j.jhep.2022.05.019
 J. Hepatol. 2022, 77, 978–990

Research Article
Viral Hepatitis

JOURNAL OF HEPATOLOGY

Mechanisms of CD8+ T-cell failure in chronic hepatitis E virus infection

Janine Kemming[1,2], Swantje Gundlach[3,4], Marcus Panning[5], Daniela Huzly[5], Jiabin Huang[3,4], Marc Lütgehetmann[3,4], Sven Pischke[4,5], Julian Schulze zur Wiesch[4,6], Florian Emmerich[7], Sian Llewellyn-Lacey[8], David A. Price[8,9], Yakup Tanriver[10], Klaus Warnatz[11], Tobias Boettler[1], Robert Thimme[1], Maike Hofmann[1], Nicole Fischer[3,4], Christoph Neumann-Haefelin[1,*]

[1]Department of Medicine II (Gastroenterology, Hepatology, Endocrinology and Infectious Diseases), Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [2]Faculty of Biology, University of Freiburg, Freiburg, Germany; [3]Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany; [4]German Center for Infection Research (DZIF), Hamburg-Lübeck-Borstel-Riems, Germany; [5]Institute of Virology, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [6]Department of Medicine, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; [7]Institute for Transfusion Medicine and Gene Therapy, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [8]Division of Infection and Immunity, Cardiff University School of Medicine, Cardiff, United Kingdom; [9]Systems Immunity Research Institute, Cardiff University School of Medicine, Cardiff, United Kingdom; [10]Department of Medicine IV (Nephrology and Primary Care), Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [11]Department of Rheumatology and Clinical Immunology, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

See Editorial, pages 909–911

Background & Aims: In immunosuppressed patients, persistent HEV infection is common and may lead to cirrhosis and liver failure. HEV clearance depends on an effective virus-specific CD8+ T-cell response; however, the knowledge gap around HEV-specific CD8+ T-cell epitopes has hindered analysis of the mechanisms of T-cell failure in persistent infection.

Methods: We comprehensively studied HEV-specific CD8+ T-cell responses in 46 patients with self-limiting (n = 34) or chronic HEV infection (n = 12), by epitope-specific expansion, functional testing, *ex vivo* peptide HLA class I tetramer multi-parametric staining, and viral sequence analysis.

Results: We identified 25 HEV-specific CD8+ T-cell epitopes restricted by 9 different HLA class I alleles. In self-limiting HEV infection, HEV-specific CD8+ T cells were vigorous, contracted after resolution of infection, and formed functional memory responses. In contrast, in chronic infection, the HEV-specific CD8+ T-cell response was diminished, declined over time, and displayed phenotypic features of exhaustion. However, improved proliferation of HEV-specific CD8+ T cells, increased interferon-γ production and evolution of a memory-like phenotype were observed upon reduction of immunosuppression and/or ribavirin treatment and were associated with viral clearance. In 1 patient, mutational viral escape in a targeted CD8+ T-cell epitope contributed to CD8+ T-cell failure.

Conclusion: Chronic HEV infection is associated with HEV-specific CD8+ T-cell exhaustion, indicating that T-cell exhaustion driven by persisting antigen recognition also occurs in severely immunosuppressed hosts. Functional reinvigoration of virus-specific T cells is at least partially possible when antigen is cleared. In a minority of patients, viral escape also contributes to HEV-specific CD8+ T-cell failure and thus needs to be considered in personalized immunotherapeutic approaches.

Lay summary: Hepatitis E virus (HEV) infection is usually cleared spontaneously (without treatment) in patients with fully functioning immune systems. In immunosuppressed patients, chronic HEV infection is common and can progress rapidly to cirrhosis and liver failure. Herein, we identified the presence of HEV-specific CD8+ T cells (a specific type of immune cell that can target HEV) in immunosuppressed patients, but we show that these cells do not function properly. This dysfunction appears to play a role in the development of chronic HEV infection in vulnerable patients.

Introduction

Ten to twenty percent of individuals in Europe and North America are seropositive for HEV; however, only a minority of patients develop a symptomatic infection, and fulminant courses are mainly observed in the elderly and those with preexisting liver disease.[1,2] While HEV infections are self-limiting in immunocompetent hosts, infections with HEV genotype 3, the predominating HEV genotype in Europe and North America, persist in 50-70% of immunosuppressed patients.[1,2] Chronic infection is defined as HEV replication for >3 months, since no spontaneous HEV clearance was observed after this period. In patients with chronic HEV infection, reduction of immunosuppression as well as ribavirin treatment

Keywords: CD8+ T-cell response; antiviral immunity; immunological memory; viral escape; T-cell exhaustion.
Received 16 November 2021; received in revised form 11 May 2022; accepted 16 May 2022; available online 28 May 2022

* Corresponding author. Address: Department of Medicine II, Freiburg University Medical Center, Hugstetter Straße 55, 79106 Freiburg, Germany.
E-mail address: christoph.neumann-haefelin@uniklinik-freiburg.de (C. Neumann-Haefelin).

https://doi.org/10.1016/j.jhep.2022.05.019

may result in viral clearance and are recommended by EASL guidelines.[1] However, approximately 10% of patients do not respond to this treatment strategy, and there are currently no further treatment options available.[1] In a substantial number of patients, chronic HEV infection progresses rapidly towards cirrhosis and liver failure. Thus, novel treatment options including immunotherapeutic strategies are urgently needed.

Recent *in vivo* evidence from 2 animal models (chicken and rhesus macaque) demonstrates an important role of HEV-specific CD8+ T cells in HEV control.[3,4] However, in humans, there is still a paucity of data on HEV-specific CD8+ T-cell immunity.[5] Studies using recombinant HEV capsid protein (corresponding to open reading frame [ORF] 2) as well as overlapping peptides covering some or all HEV domains (ORF1-3) showed broad and vigorous HEV-specific CD8+ T-cell responses in immunocompetent patients with acute infection that contracted upon resolution of infection.[6–10] However, immunosuppressed patients with chronic HEV infection displayed a narrow and weak HEV-specific CD8+ T-cell response mostly at or below the limit of detection. *In vitro* checkpoint inhibition targeting programmed cell death 1 (PD1) or cytotoxic T lymphocyte antigen-4 (CTLA-4), as well as successful antiviral treatment, partially restored HEV-specific CD8+ T-cell responses in some patients,[9,11] however, checkpoint inhibition is not a suitable treatment strategy in transplanted patients after failed ribavirin treatment, since it may induce transplant rejection. It is thus necessary to more precisely define the mechanisms of HEV-specific CD8+ T-cell failure in persistent HEV infection. These studies have been limited, however, by the paucity of described HEV-specific CD8+ T-cell epitopes that can be analyzed, *e.g.* using peptide/HLA class I multimer technology. Indeed, to date, only 3 HLA-A*02-restricted HEV-specific CD8+ T-cell epitopes have been defined and fine-mapped.[12]

Thus, we set out to define a broad range of HEV-specific CD8+ T-cell epitopes restricted by common HLA class I alleles in acute-resolving HEV infection; we then used these novel HEV-specific CD8+ T-cell epitopes to analyze the mechanisms of HEV-specific CD8+ T-cell failure in a cohort of immunocompromised patients with chronic HEV infection.

Patients and methods

Study cohort

A total of 34 patients with self-limiting HEV infection and 12 patients with chronic HEV infection were included. All donors were recruited at the Freiburg University Medical Center, Germany. Acute HEV infection was determined by positive plasma HEV RNA, whereas resolved infection was defined by negative HEV RNA testing and the detection of anti-HEV IgG and/or IgM. Chronic HEV infection was defined as positive HEV RNA for >3 months according to EASL guidelines. Patient characteristics are displayed in Table 1. Next-generation sequencing was used for HLA-typing. Written informed consent was obtained in all cases and the study was conducted according to federal guidelines, local ethics committee regulations (Albert-Ludwigs-Universität, Freiburg, Germany; vote #: 437/18), and the Declaration of Helsinki (1975).

Statistics

Statistical analysis was performed with GraphPad Prism 8 (USA). Statistical significance was assessed by Kruskal-Wallis testing including Dunn's multiple comparisons test and Mann-Whitney testing. (*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001).

Immunological and sequencing techniques are described in the supplementary methods section.

Results

Definition of virus-specific CD8+ T-cell epitopes in patients with self-limiting HEV infection

To define HEV-specific CD8+ T-cell epitopes, we predicted 58 HEV-specific epitopes restricted by 10 common HLA class I alleles *in silico* (Table S1). We tested these epitope peptides using peripheral blood mononuclear cells (PBMCs) from 34 patients with self-limiting HEV infection expressing the respective HLA alleles (Table 1). Since results were similar for patients with acute-resolving (n = 24) and resolved HEV infection (n = 10), these patients were analyzed together. After antigen-specific expansion for 14 days, we could detect interferon-γ (IFNγ)-producing CD8+ T cells in response to peptide stimulation in 29/34 (86%) patients with self-limiting HEV infection (Fig. 1A; compare Fig. 1B for representative dot blots and Fig. S1A for negative controls). Single patients recognized up to 8 epitopes, while the majority of patients recognized 1-2 epitopes (Fig. 1A). Overall, 25/58 predicted epitopes were confirmed experimentally (Fig. 1C). 17/25 (68%) confirmed epitopes were located within ORF1, reflecting its overall length, while the remaining epitopes were located within ORF2 (Fig. 1D). Of note, the 78 positive responses were distributed quite equally to ORF1 and ORF2, indicating a relative immunodominance of the smaller ORF2. The confirmed epitopes were restricted by all HLA alleles included in the epitope prediction except for HLA-B*07 (Fig. 1E). Strikingly, approx. 25% of confirmed epitopes and 50% of responses were restricted by HLA-A*02:01, although the cohort was not preselected for HLA-A*02:01 expression. IFNγ-producing HEV-specific CD8+ T cells could also be detected directly *ex vivo* by Elispot analysis in PBMCs from 3 donors with acute HEV infection (Fig. S1B,C).

Effector function and memory formation in self-limiting HEV infection

To further characterize the HEV-specific CD8+ T-cell response in self-limiting HEV infection, we applied a peptide/HLA class I (pHLA-I) tetramer-based *ex vivo* enrichment strategy (gating strategy displayed in Fig. S2). HEV-specific CD8+ T cells targeting the 4 epitopes analyzed were readily detectable (Fig. 2A); their frequency did not vary by targeted epitope but differed between patients with acute *vs.* resolved HEV infection (Fig. 2B). During acute infection, the frequency of HEV-specific CD8+ T cells was similar compared to influenza infection and severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection but significantly higher compared to acute HBV infection (Fig. 2C). In line with the formation of a potent effector CD8+ T-cell response, we detected high expression of the activation marker CD38, the proliferation marker Ki67, the effector molecule granzyme B (GranB) and the transcription factor Tbet (also known as TBX21) (Fig. 2D). Expression of the memory marker CD127 was still low on HEV-specific CD8+ T cells (Fig. 2D). In an HLA-A*02:01+/B*27:05+ individual, we longitudinally monitored the differentiation of HEV-specific CD8+ T cells from early acute infection to >4 years after viral clearance. Upon viral elimination, the A*02/ORF2$_{493\text{-}501}$ and B*27/ORF1$_{1315\text{-}1323}$-specific CD8+ T-cell response contracted with frequencies declining by up to 2 logs within the first months, but then plateaued throughout follow-up (Fig. 2E+F; exemplary *ex vivo* tetramer stainings without

Table 1. Patient characteristics.

Patient code	Age (years)*	Sex	HLA type	Anti-HEV IgG (AU/ml)#*	Anti-HEV IgM (AU/ml)#*	HEV RNA max (IU/ml)	ALT max (U/L)	Bilirubin max (mg/dl)	Comment/comorbidity	Immunosuppression	Therapy
Patients with self-limiting infection											
aHEV-1	51	M	A*02:01, A*26:01, B*08:01, B*27:05	55.6	145	16,031	2,677	1.8	Eosinophilic granulomatosis with polyangiitis	–	–
aHEV-2	79	M	A*01, A*03, B*08, B*35	93.7	157	16,031	3,883	7.8	First diagnosis of CUP (small-cell carcinoma)	–	–
aHEV-3	51	M	A*02, A*29, B*27, B*51	117	140	68,702	1,215	0.9	NSCLC, infection during chemotherapy	–	–
aHEV-4	69	M	A*02:01, A*11:01, B*07:02, B*14:02	112	155	119,467	5,281	7		–	–
aHEV-5	55	F	A*03:01, A*69:01, B*18:01, B*44:02	25.2	228	1,059,862	2,210	2.4	Diabetes mellitus II	–	–
aHEV-6	50	M	A:01:01, A*02:01, B*07:01	110	168	33,187	1,057	1.1	Gluten-sensitive enteropathy	–	–
aHEV-7	57	M	A*01:01, A*24:02, B*18:01, B*35:08	141	254	511,976	1,993	22.6	Squamous cell carcinoma	–	–
aHEV-8	20	F	A*01:01, A*03:01, B*07:02, B*55:01	137	217	609,495	1,606	4		–	–
aHEV-9	26	M	A*01:01, A*02:01, B*08:01, B*15:01	83.5	164	354	1,629	1.1		–	–
aHEV-10	71	M	A*02:01, A*30:01, B*13:02, B*15:01	36.8	140	294,422	2,642	10.4	Diabetes mellitus II	–	–
aHEV-11	54	M	A*02:01, B*40:01, B*44:27	109	175	889,000	5,559	21.7	Acute on chronic liver failure (cryptogenic cirrhosis)	–	–
aHEV-12	80	F	A*11:01, A*68:02, B*14:02, B*35:01	5.80	139	2,050,000	979	1	Ovarial carcinoma	–	–
aHEV-13	64	F	A*02:01, A*03:01, B*15:01, B*35:08	154	157	3,142	825	0.8	H/o AML, h/o allogeneic PBSCT 01/13	–	–
aHEV-14	21	M	A*02, A*33, B*44, B*51	206	137	247,314	3,710	9.4	HEV genotype 1 (India)	–	–
aHEV-15	36	F	A*02:01, A*11:01, B*07:02, B*40:01	22.9	152	334,550	938	0.8	Mamma carcinoma, infection during chemotherapy	–	–
aHEV-16	58	F	A*01:01, A*02:01, B*08:01, B*38:01	26.5	153	598,000	2,039	4.8	Allergic asthma	–	–
aHEV-17	57	M	A*25:01, A*31:01, B*18:01, B*27:05	122	164	142,223	1,546	1.7		–	–
aHEV-18	49	M	A*02:01, A*68:01, B*07:02, B*40:01	90.5	59.5	33,187	344	0.2	HEV-associated neuralgic amyotrophy	–	–
aHEV-19	56	M	A*02:01, A*02:35, B*07:02, B*44:02	140	174	873	889	1.6	Alcohol-related cirrhosis	–	–
aHEV-20	50	M	A*02:01, B*08:01, B*45:01	122	192	1,807	1,410	3.4		–	–
aHEV-21	28	F	A*02:01, A*29:02, B*07:02, B*51:01	15.8	93.5	949,000	1,247	1.2	Mild leukopenia with mildly reduced antibodies	–	–
aHEV-22	59	F	A*02:01, A*11:01, B*18:01, B*44:02	165	160	ND	306	1.4	H/o liver transplantation; documented seroconversion	–	–
aHEV-23	61	M	A*02:01, B*51:01, B*51:XX	ND	ND	31,000	1,059	6.2	First diagnosis of sarcoidosis	–	–
aHEV-24	65	M	A*01:01, A*02:01, B*40:01, B*51:01	140	143	420,000	1,248	11	Sarcoidosis	–	–
rHEV-1	46	F	A*02:01, B*40:01, B*44:02	116	53.9	neg	34	0.3	Myocarditis	–	–
rHEV-2	32	F	A*02:01, A*32:01, B*40:02, B*44:02	152	31.2	neg	44	0.6	Adenoviral tonsilitis	–	–
rHEV-3	52	F	A*02:01, A*24:02, B*18:01, B*41:01	124	24.4	neg	22	0.2	Cervical disc prolapse	–	–
rHEV-4	59	M	A*02:01, A*11:01, B*44:02, B*51:01	77.9	9.2	ND	27	0.4	Idiopathic hepatopathy	–	–
rHEV-5	90	F	A*02:01, A*03:01, B*35:01, B*40:01	25.9	6.7	ND	810	1.9	Drug-induced hepatopathy	–	–
rHEV-6	52	F	A*02:01, A*03:01, B*13:02, B*15:01	269	46.4	neg	141	17.8	Drug-induced hepatopathy	–	–
rHEV-7	60	F	A*01, A*11, B*08, B*35	40.8	7.7	ND	16	0.2	Chronic HBV infection	–	–
rHEV-8	54	F	A*02:01, A*33:03, B*35:01, B*44:05	82	9	ND	36	0.5	NASH	–	–
rHEV-9	52	M	A*03:01, A*31:01, B*15:01, B*27:05	181	4.5	ND	91	1.1	Autoimmune hepatitis	–	–
rHEV-10	62	F	A*01:01, A*24:02, B*38:01, B*57:01	121	162	neg	22	0.3	H/o PBSCT for AML, h/o HBV reactivation	–	–

(*continued on next page*)

Table 1. (*continued*)

Patient code	Age (years)*	Sex	HLA type	Anti-HEV IgG (AU/ml)#*	Anti-HEV IgM (AU/ml)#*	HEV RNA max (IU/ml)	ALT max (U/L)	Bilirubin max (mg/dl)	Comment/comorbidity	Immunosuppression	Therapy
Patients with chronic infections											
cHEV-1	24	M	A*02:01, A*03:01, B*35:01, B*39:01	ND	ND	511,976	534	3.8	Liver transplantation (3x), kidney transplantation	TAC/MMF/PRED	RBV 11w
cHEV-2	46	F	A*01:01, A*11:01, B*08:01, B*44:02	1.6	1.8	4,542,023	710	0.9	Autologous PBSCT for B-non-Hodgkin lymphoma)	Rituximab	RBV 16w
cHEV-3	56	M	A*02:01, A*25:01, B*15:01, B*18:01	158	152	4,542,023	332	1.6	Kidney transplantation	TAC/MMF/PRED	RBV 11w
cHEV-4	63	M	A*02, B*08, B*51	ND	ND	19,464,771	81	0.5	Kidney transplantation	TAC/MMF/PRED	RBV 11w
cHEV-5	52	F	A*01:01, A*24:02, B*08:01, B*40:02	152	134	9,402,629	131	2.4	Kidney transplantation	TAC/MMF/PRED	RBV 20w + 21w
cHEV-6	58	F	A*02:01, A*24:02, B*07:02, B*40:01	171	282	115,000	45	0.6	Kidney transplantation	TAC/MMF/PRED	RBV 20w
cHEV-7	53	M	A*02, B*15:01, B*44	1.9	2.4	pos	340	0.7	Lung transplantation, primary cerebral lymphoma, autologous PBSCT	TAC/EVR/PRED	RBV 47w
cHEV-8	58	M	A*02:01, A*03:01, B*07:02, B*35:01	ND	ND	363,363	124	0.5	Kidney transplantation	TAC/MMF/PRED	RBV 12w
cHEV-9	74	M	A*01:01, A*32:01, B*08:01, B*15:01	176	86	8,290,000	508	1.5	Sero-negative erosive polyarthritis	Abatacept	Abatacept paused
cHEV-10	65	M	A*01:01, A*03:01, B*35:01, B*57:01	ND	ND	6,127,500	181	0.6	Autologous PBSCT (3x) for multiple myeloma	CyA/MMF/ATG	RBV 12w
cHEV-11	18	M	A*02, B*15:01	neg	neg	25,140,000	688	1.2	Allogenic (HLA-identical) PBSCT for pre-T-ALL	Rituximab	none
cHEV-12	55	F	A*11:01, A*24:02, B*15:02, B*35:01	ND	ND	267,000	84	0.5	Kidney transplantation	TAC/MMF/PRED	none

aHEV, acute HEV; AML, acute myeloid leukemia; ATG, antithymocyte globulin; AU, arbitrary units; cHEV, chronic HEV; CUP, cancer of unknown primary; CyA, cyclosporin A; EVR, everolimus; MMF, mycophenolate mofetil; NASH, non-alcoholic steatohepatitis; ND, not done; NSCLC, non-small cell lung cancer; PBSCT, peripheral blood stem cell transplant; PRED, prednisone; RBV, ribavirin; rHEV, resolved HEV; TAC, tacrolimus; T-ALL, T-cell acute lymphoblastic leukemia.
*At first diagnosis.
#cut-off 20 AU/ml.

enrichment can be found in Fig. S3). Multi-dimensional scaling analysis of the longitudinal B*27/ORF1$_{1315-1323}$ -specific CD8+ T-cell response in this individual revealed that early time points cluster away from each other while late time points cluster together, which emphasizes the early dynamic differentiation patterns during acute infection followed by the establishment of a stable memory cell pool (Fig. 2G). Diffusion map embedding, incorporating longitudinal flow cytometry data from B*27/ORF1$_{1315-1323}$-specific CD8+ T cells, revealed that CD8+ T cells from the earliest and latest time points localized at opposing ends of the trajectory (Fig. 2H). Upon resolution of acute infection, expression of CD38 and Ki67 was lost and a memory response formed as characterized by expression of CD127 and T-cell factor 1 (TCF1) (Fig. 2H+I). We also found a tendency towards lower expression of GranB and PD1 on HEV-specific CD8+ T cells of patients with resolved compared to acute infection (Fig. 2I). The memory cell pool in resolved HEV infection was largely composed of the CD45RA$^-$/CCR7$^-$ effector memory subset with a minority of terminally differentiated effector memory cells re-expressing CD45RA and few CCR7$^+$ central memory CD8+ T cells (Fig. 2J). Collectively, these data indicate that acute/self-limiting HEV infection is associated with a strong HEV-specific effector CD8+ response that evolves into a *bona fide* memory response after resolution.

Functional CD8+ T-cell responses in chronic HEV infection are lost over time but can be partially reinvigorated by treatment-induced viral clearance

We next tested the 58 epitope candidates in 12 patients with chronic HEV infection. These 12 patients included 8 patients with solid organ transplants, 3 patients with autologous or HLA-identical allogeneic peripheral blood stem cell transplants, and 1 patient treated with the CTLA-4 agonist abatacept for erosive polyarthritis (see Table 1 for detailed patient characteristics). After peptide-specific expansion for 14 days, we found IFNγ-producing HEV-specific CD8+ T cells in only 4/12 (33%) patients with chronic infection (Fig. 3A,B) compared to 29/34 (86%) in self-limiting infection (p = 0.0006). In these 4 patients, a total of 7 responses (1-2 per patient) targeted 6 epitopes. Of these 6 epitopes, we had previously identified 4 in self-limiting HEV infection (3 restricted by HLA-A*02:01, 1 by HLA-A*11:01). 2 novel HLA-B*35:01 restricted epitopes only tested positive in 1 patient with chronic infection each. All epitopes were located in ORF1 except for A*02/ORF2$_{493-501}$ (Fig. 3C). Similar to self-limiting infection, the majority of responses (4/7) were restricted by HLA-A*02:01 (Fig. 3D). Interestingly, when an IFNγ response was observed in response to peptide stimulation, it was of a comparable magnitude to those in self-limiting HEV infection (Fig. 3E). Elimination of chronic HEV infection was achieved in all patients upon lowering of immunosuppression and/or ribavirin therapy (Table 1). 11/12 patients were re-tested for IFNγ responses upon resolution of infection; 1 patient was lost to follow-up for immunological analysis. Notably, 4 patients without a pre-treatment IFNγ response developed HEV-specific responses upon resolution of infection (Fig. 3F). For example, patient cHEV-1, who was infected for >4 years, developed HEV-specific IFNγ responses against 4 epitopes upon ribavirin-associated viral clearance (Fig. 3G). When we arranged all chronically infected patients on a timeline according to the duration of infection, it became obvious that CD8+ T-cell

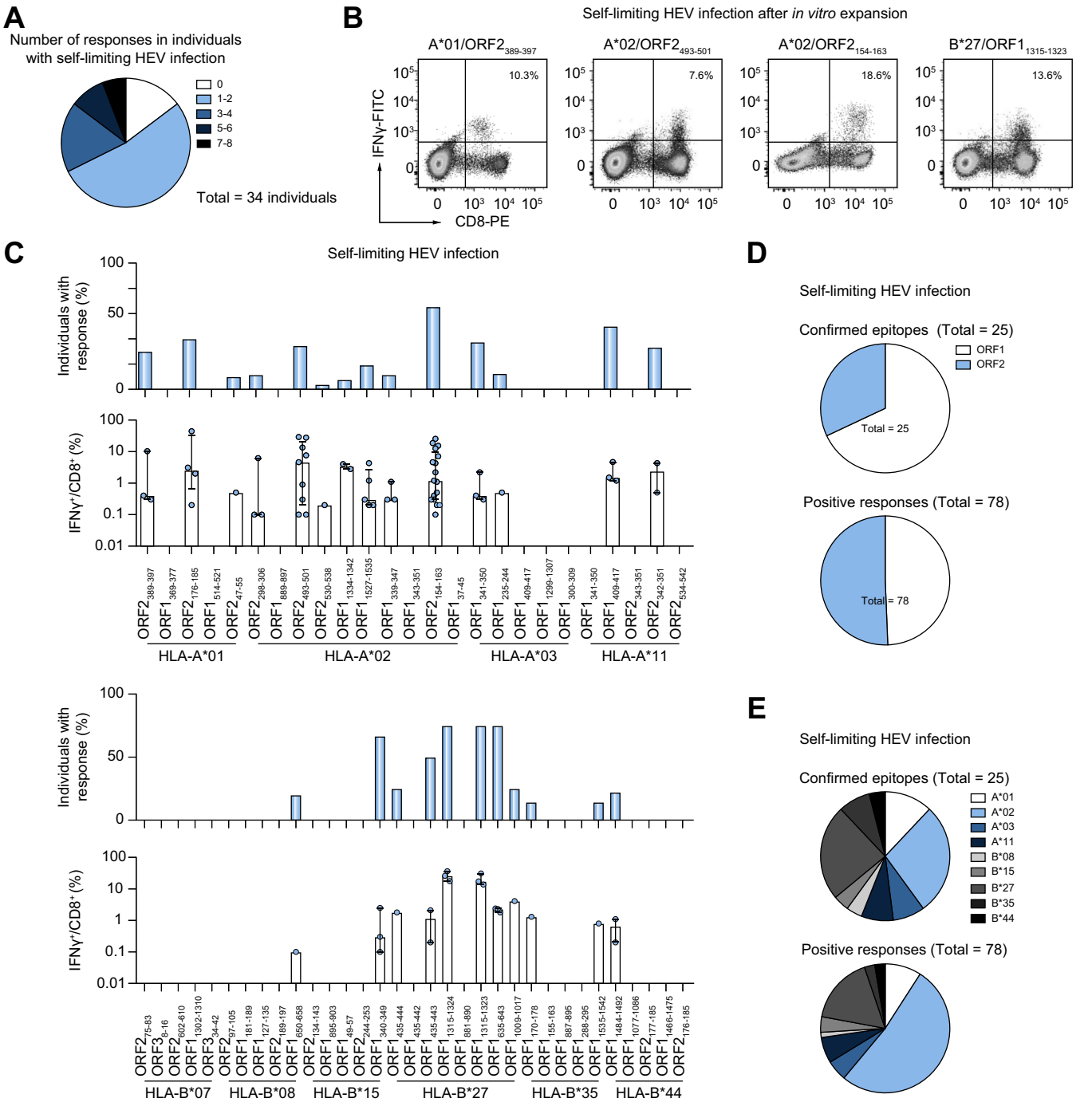


Fig. 1. Definition of virus-specific CD8+ T-cell epitopes in patients with self-limiting HEV infection. (A) Number of IFNγ responses in 34 individuals with self-limiting HEV infection after *in vitro* expansion. (B) Exemplary dot plots of positive IFNγ responses after re-stimulation with the indicated epitope peptides after 14 days *in vitro* culture. Percentages indicate IFNγ-positive CD8+ T cells of total CD8+ T cells. (C) Percentages of individuals with self-limiting HEV infection with positive IFNγ response targeting a given *in silico* predicted HLA-A- or HLA-B-restricted CD8+ T-cell epitope and strengths of individual responses. (D) Distribution of confirmed epitopes and positive responses targeting ORF1 or ORF2. (E) HLA restriction of confirmed epitopes and positive responses. ORF, open reading frame.

responses were lost over time during persistent infection. However, the partial reinvigoration of these responses upon treatment-induced viral elimination seemed to be independent of the duration of infection (Fig. 3H). Of note, while the number of CD8+ T-cell responses in chronic infection was significantly diminished compared to acute infection (p = 0.0172), responses were readily detectable after treatment-associated resolution to a level observed in resolved infection (Fig. 3I; p >0.9999). Collectively, these data indicate that functional HEV-specific CD8+ T-cell responses are strongly diminished during the course of chronic HEV infection, but can be at least partially reinvigorated by treatment-induced viral elimination. In line

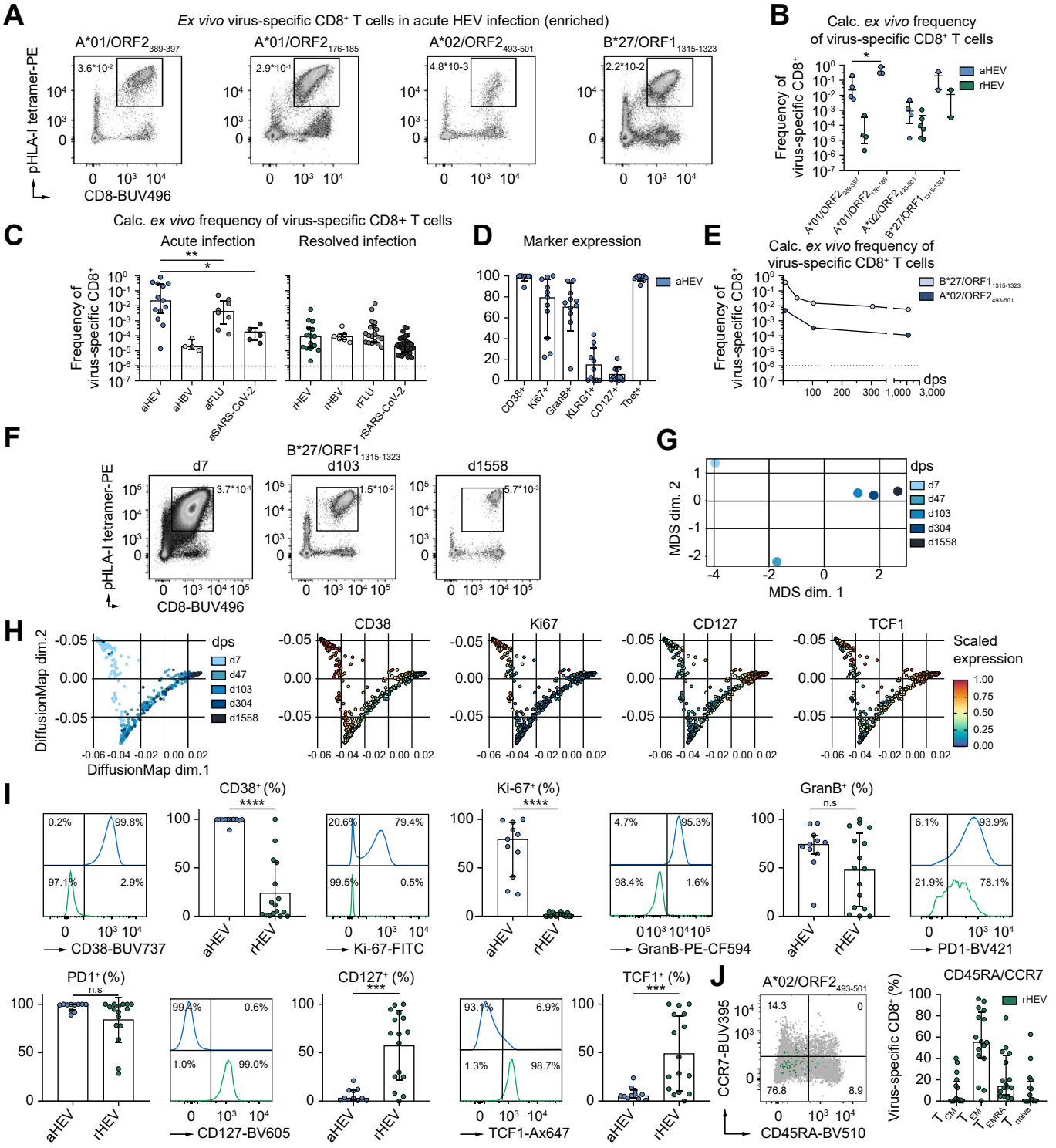


Fig. 2. Effector function and memory formation in self-limiting HEV. (A) Exemplary dot plots after *ex vivo* peptide/HLA class I tetramer enrichment using 10-20x 10^6 PBMCs in self-limiting HEV infection. (B) Calculated *ex vivo* frequency of epitope-specific CD8+ T cells in aHEV and rHEV infection. (C) Calculated *ex vivo* virus-specific CD8+ T cells in acute and resolved HEV, HBV, FLU, and SARS-CoV-2 infection. (D) Expression of CD38, Ki67, GranB, KLRG1, CD127, and Tbet in epitope-specific cells in acute HEV infection. (E) Longitudinal frequencies of B*27/ORF1$_{1315-1323}$ -specific CD8+ T cells in an individual with acute-resolving HEV infection. (F) Exemplary dot plots of longitudinal B*27/ORF1$_{1315-1323}$ -specific CD8+ T cells in an individual with acute-resolving HEV infection. (G) MDS analysis of longitudinally sampled B*27/ORF1$_{1315-1323}$ -specific CD8+ T cells. (H) Diffusion map showing flow cytometry data of B*27/ORF1$_{1315-1323}$ -specific CD8+ T cells in relation to dps. Protein expression levels of CD38, Ki67, CD127 and TCF1 are plotted on the diffusion map. (I) Exemplary histograms and marker expression patterns in HEV-specific CD8+ T cells in acute compared to resolved HEV infection. (J) Exemplary dot plot and overall expression of CD45RA and CCR7 in epitope-specific CD8+ T cells in resolved HEV infection. Bar charts show median with IQR. Kruskal-Wallis with rank-sum tests including Dunn's multiple comparisons or Mann-Whitney testing were performed. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001. aHEV, acute HEV; dps, days post symptom onset; FLU, influenza; MDS, multi-dimensional scaling; PBMCs, peripheral blood mononuclear cells; rHEV, resolved HEV.

with these observations, frequencies of HEV-specific CD8+ T cells were too low to be detected by *ex vivo* IFNγ Elispot analysis in 4 chronically infected patients at RNA+ and RNA- time points (data not shown), with the exception of donor cHEV-5, in whom *ex vivo* IFNγ responses against 2 HLA-A*01:01 restricted epitopes could be detected only upon resolution of infection (Fig. S1D). Peak alanine aminotransferase (ALT) and viral load differed significantly between acute and chronic HEV infection (Fig. S4A), but did not correlate with each other (Fig. S4B). Of note, no correlation between peak ALT or viral load and the number of targeted CD8+ T-cell epitopes was found in acute infection (Fig. S4C/D, left panels). In chronic infection, however, peak ALT and viral load correlated negatively with the number of HEV-specific CD8+ T-cell responses (Fig. S4C/D, right panels).

HEV-specific CD8+ T cells in chronic HEV infection are rare but functional and exhibit a memory-like phenotype after viral resolution

To characterize the CD8+ T-cell response in chronic HEV infection in more detail, we used PBMCs from 9 HLA-A*01:01+ (n = 4) or HLA-A*02:01+ (n = 5) patients during chronic infection and after treatment-induced resolution to enrich either A*01/ORF2$_{389-397}$ or A*02/ORF2$_{493-501}$–specific CD8+ T cells *ex vivo* (Fig. 4A, patients with positive responses at one or both time points are displayed; sampling time points and course of infection are depicted in Fig. S5). 2/4 HLA-A*01:01+ and 2/5 HLA-A*02:01+ patients had detectable epitope-specific CD8+ T-cell responses during chronic infection. After resolution, an additional HLA-A*01:01+ and an HLA-A*02:01+ patient each developed positive responses (Fig. 4B). The *ex vivo* frequency of epitope-specific CD8+ T cells was not significantly different between chronic, resolved, and treated chronic HEV infection but was significantly lower compared to acute HEV infection (Fig. 4C). Using longitudinal samples of the 4 patients displaying responses during chronic and resolved infection (cHEV-2, cHEV-3, cHEV-9, cHEV-11), as well as late samples from the 2 patients who developed responses after resolution (cHEV-5, cHEV-6), we analyzed the evolution of the HEV-specific CD8+ T-cell phenotype before and after viral clearance (Fig. 4D,E). During chronic infection, epitope-specific CD8+ T cells expressed the activation marker CD38 and mostly displayed Ki67 as a marker of proliferation (Fig. 4E). However, they also expressed high levels of PD1 and low levels of CD127 and TCF1, with the majority of cells being in the CD127-PD1+ subset, indicating some characteristics of terminally exhausted CD8+ T cells (Fig. 4D,E). After resolution of chronic infection, CD8+ T cells displayed a memory-like phenotype (PD1 moderate, CD127+, TCF1+) with the majority of cells being CD127+PD1+ (Fig. 4D). They also lost expression of CD38 (Fig. 4E). Collectively, these data indicate that HEV-specific CD8+ T cells develop a terminally exhausted phenotype during chronic infection with albeit (partially) maintained proliferative capacity. They also indicate that a sufficient pool of memory-like CD8+ T cells remains to be restored after viral elimination.

Impact of immunosuppression on the HEV-specific CD8+ T-cell response and impact of HEV-specific CD8+ T-cell response on viral control

To address the impact of immunosuppression on the induction of HEV-specific CD8+ T cells, T-cell functionality and viral titers, we longitudinally studied patient cHEV-9 (details in Table 1) from 7 days post-diagnosis of HEV infection for >2 years. At diagnosis, cHEV-9 presented with positive HEV IgG and IgM and high viral titers. We were able to characterize the A*01/ORF2$_{389-397}$ and A*01/ORF2$_{298-306}$-specific CD8+ T-cell response longitudinally (Fig. 5A,B). During abatacept treatment, the frequency of HEV-specific CD8+ T cells was low, ranging from 10^{-5}-10^{-4}. However, T-cell numbers were reinvigorated upon discontinuation of abatacept treatment. Indeed, their substantial increase in frequency directly preceded a sharp decline of viremia that resulted in viral clearance and normalization of liver parameters (Fig. 5C). Shortly after viral clearance, HEV-specific CD8+ T cells declined below the limit of detection in peripheral blood. Interestingly, on d771 post-diagnosis (d645 post-resolution), the HEV-specific CD8+ T-cell response had recovered and persisted at frequencies of approximately 10^{-5} (Fig. 5C). During abatacept treatment, HEV-specific CD8+ T cells recognized their cognate antigen (demonstrated by high expression of the activation marker CD38 [Fig. 5D]); however, they lacked GranB expression (a marker for effector function [Fig. 5E]). Upon discontinuation of abatacept, GranB expression was rescued and correlated with declining viral titers, further supporting a direct impact of immunosuppression on HEV-specific CD8+ T-cell immunity.

We were also able to longitudinally follow a kidney-transplanted patient on triple immunosuppression (cHEV-5) who relapsed after ribavirin therapy and required re-treatment with ribavirin (Fig. 5F). Importantly, this patient only displayed HEV-specific CD8+ T cells *ex vivo* (Fig. 5G) after sustained virological response following the second treatment course, further indicating a role of HEV-specific CD8+ T cells in sustained viral control. Collectively, the data from these 2 patients underline the dominant role of immunosuppression in impairing HEV-specific CD8+ T-cell responses, and also the important contribution of HEV-specific CD8+ T cells for sustained viral clearance.

Viral escape contributes to HEV-specific CD8+ T-cell failure

Finally, we addressed the role of mutational viral escape in chronic HEV infection. We thus sequenced the HEV genome via ultra-deep sequencing at early and late time points (interval of 2 months to 4 years) during chronic infection in 5 patients (Fig. 6A, Table S2). In cHEV-2, we found a mutation in epitope A*01/ORF2$_{389-397}$ that was targeted in this patient. The amino acid substitution was predicted to substantially reduce HLA-A*01:01 binding (Fig. 6B). After expansion of PBMCs from cHEV-2 with the consensus or the variant peptide for 14 days, tetramer staining revealed that PBMCs only expanded in response to stimulation with the consensus peptide (Fig. 6C). When we re-stimulated the CD8+ T cells that had been expanded with the consensus peptide with either consensus or variant peptide, we observed downregulation and internalization of the T-cell receptor (TCR) as well as an increase of GranB expression as a correlate for T-cell activation upon re-stimulation with the consensus but not the variant peptide (Fig. 6D). Next, we compared the phenotype of *ex vivo*-enriched cells from cHEV-2 (variant epitope sequence) with those from cHEV-3 (consensus epitope sequence) (Fig. 6E). Cells targeting the conserved epitope in cHEV-3 displayed a PD1^{high} phenotype with expression of CD38 as a correlate for ongoing antigen recognition, whereas cells targeting the variant epitope in cHEV-2 expressed reduced levels of PD1 and had lost CD38 expression (Fig. 6E). Finally, we performed high dimensional analysis of longitudinally enriched samples from cHEV-2 and cHEV-3 including markers of

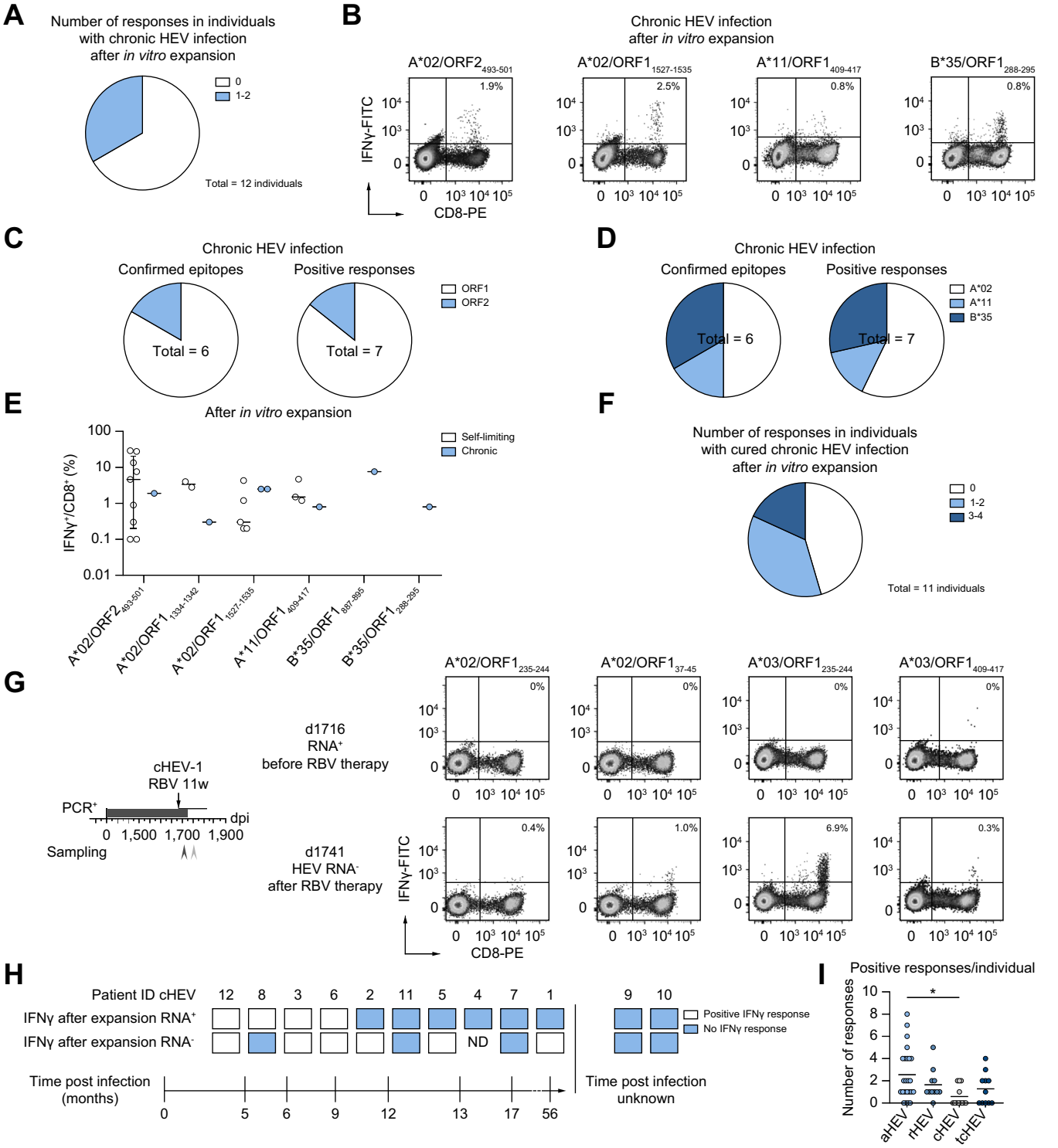


Fig. 3. Functional CD8+ T-cell responses in chronic HEV infection are lost over time but can be partially reinvigorated by treatment-induced viral clearance. (A) Number of IFNγ responses in 12 individuals with chronic HEV infection after *in vitro* expansion. (B) Exemplary dot plots of positive IFNγ responses after re-stimulation with indicated epitope peptides after 14 days *in vitro* culture. Percentages indicate IFNγ positive CD8+ T cells as a proportion of total CD8+ T cells. (C) Distribution of confirmed epitopes and positive responses targeting ORF1 or ORF2. (D) HLA restriction of confirmed epitopes and positive responses. (E) Magnitude of individual IFNγ responses in cHEV compared to aHEV infection. (F) Number of IFNγ responses in 11 individuals with cured chronic HEV infection after *in vitro* expansion. (G) Timeline depicting ribavirin treatment and sampling in patient P1. Dot plots show IFNγ responses to epitope peptides after 14 d *in vitro* expansion at the specified time points. Percentages indicate IFNγ positive CD8+ T cells of total CD8+ T cells. (H) IFNγ responses (HEV RNA+ and cured HEV RNA- time points) in chronic HEV patients plotted on a time line referring to the total duration of chronic infection f. Blue boxes indicate at least 1 positive response, white boxes indicate no response. (I) Number of positive IFNγ responses in aHEV, rHEV, cHEV, and tcHEV infection per donor with mean. Kruskal-Wallis with rank-sum test including Dunn's multiple comparisons was performed. aHEV, acute HEV; cHEV, chronic HEV; ORF, open reading frame; rHEV, resolved HEV; tcHEV, treated chronic HEV.

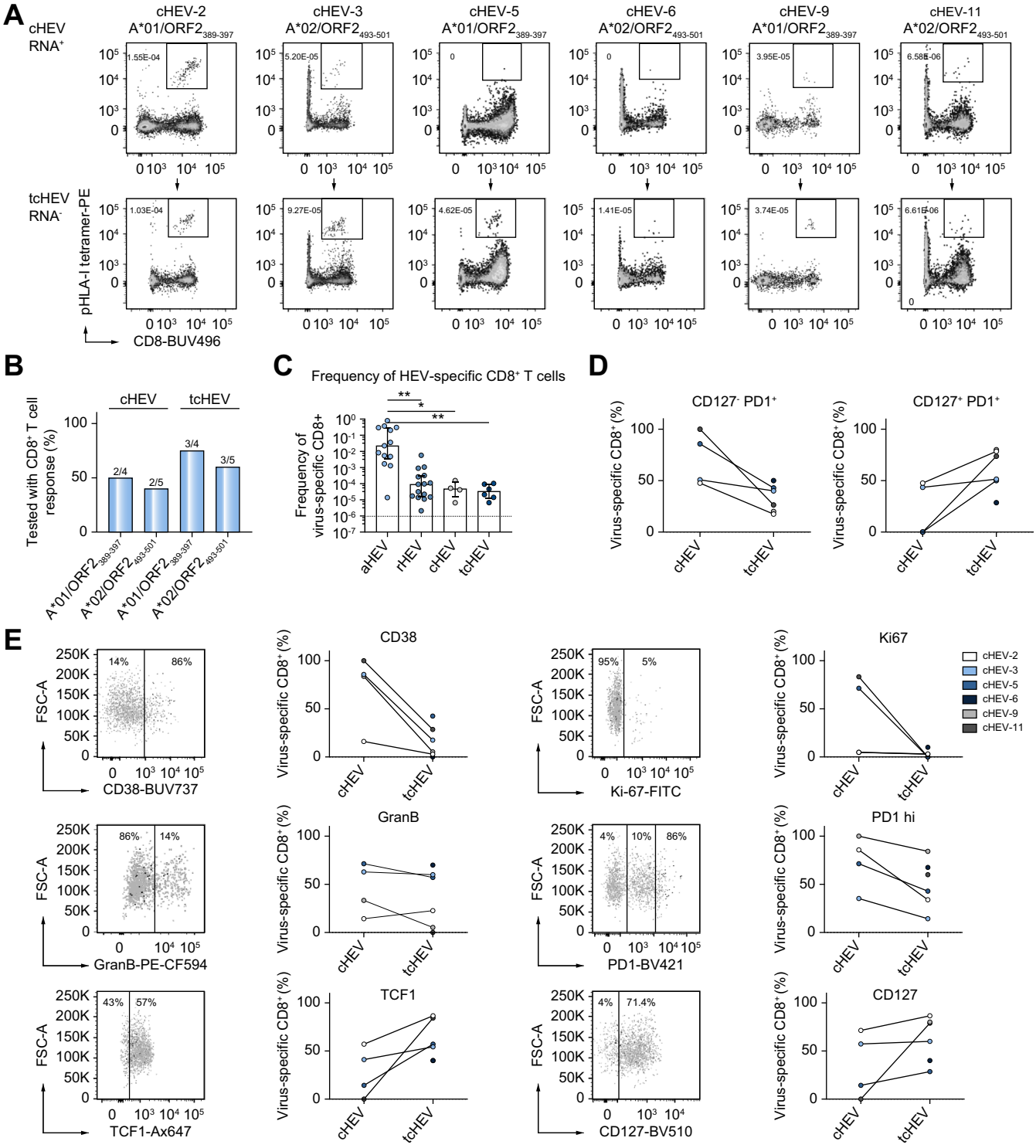


Fig. 4. HEV-specific CD8+ T cells in chronic HEV infection are rare but functional and obtain memory-like phenotype after viral resolution. (A) Dot plots after *ex vivo* tetramer enrichment using 10-20x 10^6 PBMCs in 6 patients with cHEV and tcHEV infection. (B) Percentage of tested patients with positive response in cHEV and tcHEV infection. (C) Frequencies of epitope-specific CD8+ T cells in aHEV, rHEV, cHEV and tcHEV infection. (D) Percentage of CD127-PD1+ and CD127+PD1+ virus-specific CD8+ T cells in 6 patients with cHEV and tcHEV infection. (E) Dot plots depicting protein expression levels on CD8+ bulk (grey) and epitope-specific CD8+ T cells (black) and expression of these markers in 6 patients with cHEV and tcHEV infection. Bar charts show median with IQR. Kruskal-Wallis with rank-sum tests including Dunn's multiple comparisons was performed. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001. aHEV, acute HEV; cHEV, chronic HEV; PBMCs, peripheral blood mononuclear cells; rHEV, resolved HEV; tcHEV, treated chronic HEV.

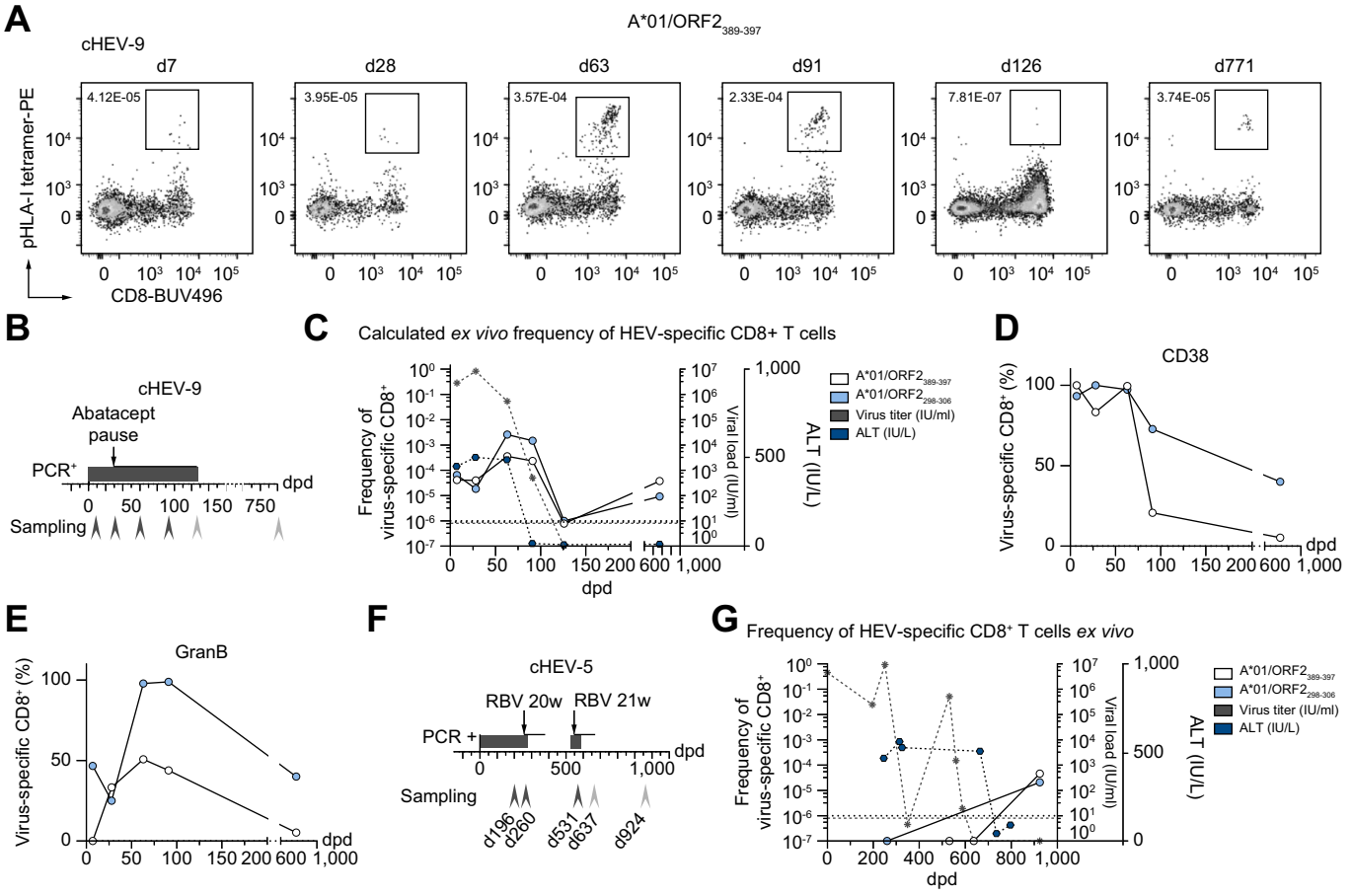


Fig. 5. Impact of immunosuppression on the HEV-specific CD8+ T-cell response and impact of HEV-specific CD8+ T-cell response on viral control. (A) Dot plots show A*01/ORF2$_{389-397}$-specific CD8+ T cells after *ex vivo* tetramer enrichment in cHEV-9 at longitudinal time points. (B) Timeline depicting sampling dates (black arrows HEV RNA+; grey arrows HEV RNA-) at dpd, abatacept pause and duration of positive HEV RNA testing (grey bar) in cHEV-9. (C) Calculated *ex vivo* frequencies of epitope-specific CD8+ T cells at indicated dpd, viral load (stars and grey dotted line) and ALT (diamond and dark blue dotted line) (D-E) Expression of CD38 and GranB of epitope-specific CD8+ T cells in patient cHEV-9 at indicated longitudinal time points. (F) Timeline depicting sampling dates (black arrows HEV RNA+; red arrows HEV RNA-) at dpd, ribavirin treatment and duration of positive HEV RNA testing (grey bar) in cHEV-5. (G) Calculated *ex vivo* frequencies of epitope-specific CD8+ T cells at indicated dpi and viral load (grey line) in cHEV-5. cHEV, chronic HEV; dpd, days post diagnosis; dpi, days post infection.

activation (Ki67, CD38, KLRG1, GranB), inhibitory receptors (PD1), memory (CD127, TCF1), and differentiation (CCR7, CD45RA, Tbet, Eomes). Multi-dimensional scaling analysis revealed that cells from cHEV-2 and cHEV-3 clustered away from each other, demonstrating their distinct differentiation. While all time points from cHEV-2 clustered together, the chronic infection time point in cHEV-3 was clearly separated from the 2 time points after resolution of infection, indicating that antigen withdrawal has an impact on the phenotype of T cells targeting conserved epitopes, but not escaped epitopes (Fig. 6F). Collectively, these data indicate that viral escape may contribute to CD8+ T-cell failure in chronic HEV infection.

Discussion

Herein, we set out to define more precisely the mechanisms of HEV-specific CD8+ T-cell failure in persistent HEV infection in immunocompromised patients. Due to the paucity of described and fine-mapped HEV-specific CD8+ T-cell epitopes,[12] we first had to define fine-mapped HEV-specific CD8+ T-cell epitopes. Using a combined *in silico* and *in vitro* approach, we defined 25 novel HEV-specific CD8+ T-cell epitopes restricted by 9 different HLA alleles. Approximately two-thirds of identified CD8+ T-cell epitopes were located in ORF1, reflecting both the relative length of the protein as well as the relative number of epitopes predicted in comparison to ORF2 and ORF3 (Fig. 1D). However, in agreement with previous reports,[7] approximately 50% of HEV-specific CD8+ T-cell responses targeted ORF2, indicating that ORF2 is a relatively immunodominant CD8+ T-cell target. Nearly one-quarter of the identified epitopes were restricted by 2 HLA types each, HLA-A*02 and HLA-B*27 (Fig. 1E). This over-representation is partially based on our selection of epitope candidates: Based on our previous experiences, we selected 10 epitope candidates for HLA-A*02 and 8 epitope candidates for HLA-B*27, compared to 5 epitope candidates for the remaining HLA alleles. Even more prominently, however, approximately 50% of positive CD8+ T-cell responses were restricted by HLA-A*02. This dominant targeting of HLA-A*02-restricted epitopes may also be explained by the high proportion of HLA-A*02+ patients in our cohort (25/34 patients with self-limiting infection). Importantly, our cohort was not pre-selected to contain a high number of HLA-A*02+ patients. Thus, HLA-A*02 may be associated with a symptomatic course of acute HEV infection;

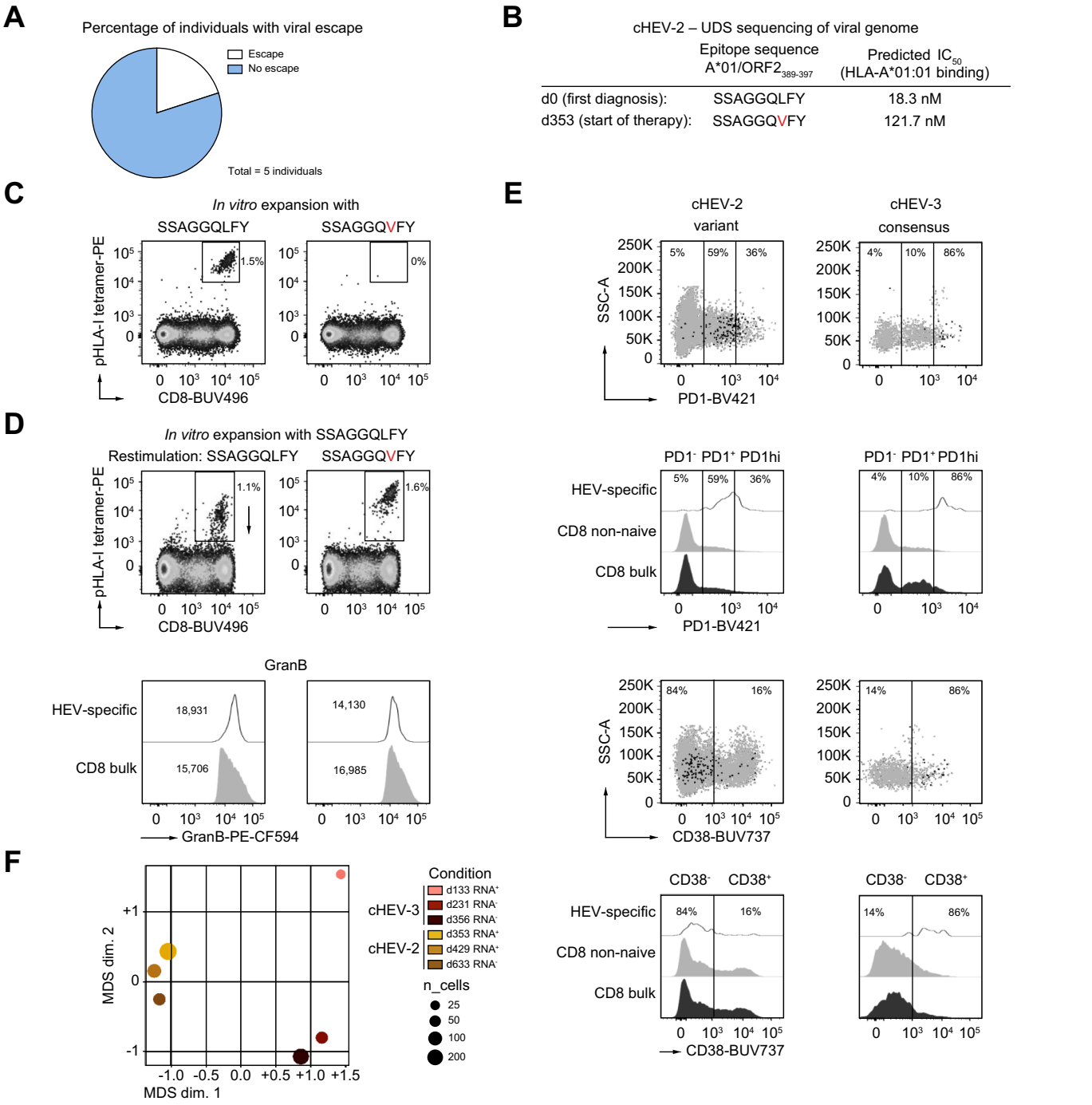


Fig. 6. Viral escape contributes to HEV-specific CD8+ T-cell failure. (A) Percentage of individuals with viral escape. (B) Longitudinal UDS data identifying viral escape variant L395V in epitope A*01/ORF2$_{389-397}$ in cHEV-2. (C) Tetramer staining after 14 days *in vitro* expansion with consensus or variant A*01/ORF2$_{389-397}$ epitope. (D) Tetramer staining after 14 days *in vitro* expansion with consensus A*01/ORF2$_{389-397}$ epitope and re-stimulation with consensus or variant peptide and histograms showing GranB expression of epitope-specific CD8+ T cells (black line) and CD8+ bulk T cells (grey). (E) Dot plots (grey: CD8+ bulk; black: antigen-specific) and histograms showing PD1 and CD38 expression of epitope-specific CD8+ T cells targeting a conserved (cHEV-3) or escaped (cHEV-2) epitope after *ex vivo* tetramer enrichment. (F) MDS analysis comparing longitudinal flow data from conserved and escaped epitopes. cHEV, chronic HEV; MDS, multi-dimensional scaling; ORF, open reading frame; UDS, ultra-deep sequencing.

however, additional studies are required to address this interesting issue.

Importantly, using our selection of predicted epitopes, the large majority (29/34) of patients with self-limited HEV infection displayed HEV-specific CD8+ T-cell responses, confirming previous reports on the immunogenicity of HEV.[5–10] Four of the 5 patients with self-limited HEV infection who did not display a response, at least to the epitopes analyzed here, had significant cancer or infectious comorbidities that may have impacted the virus-specific immune response. The only patient who lacked

both a detectable T-cell response and substantial comorbidity suffered from neuralgic amyotrophy and Guillain Barré syndrome. This intriguing finding, although limited to just 1 patient, may indicate that HEV-associated Guillain Barré syndrome is mediated by an immune mechanism independent of HEV-specific CD8+ T cells. Importantly, we expanded on previous studies regarding the HEV-specific CD8+ T-cell response in acute infection by making 2 important observations on the single-epitope level: First, in the context of acute infection, HEV-specific CD8+ T cells display a high *ex vivo* frequency, substantially and significantly outnumbering virus-specific CD8+ T cells in acute HBV infection, and numerically even outnumbering influenza- and SARS-CoV-2-specific CD8+ T cells in acute infection (Fig. 2C). Impressively, epitope-specific CD8+ T cells comprised up to one-third of the total circulating CD8+ T-cell count in some patients. These data may also partially explain why acute HEV infection can have a fulminant course in vulnerable patients, such as elderly individuals and patients with preexisting liver disease.[13] The epitopes identified in our study may help to further address this important issue in future studies. Second, by longitudinal phenotypical analysis, we demonstrate that during acute infection, HEV-specific CD8+ T cells display a highly activated effector memory phenotype. After viral clearance, the HEV-specific CD8+ T-cell pool contracts as expected, and a typical memory phenotype develops, indicating that HEV-specific CD8+ T-cell immunity in acute-resolving infection resembles influenza-specific immunity.

Next, we analyzed 12 immunocompromised patients with chronic HEV infection using the same set of epitope peptides. In agreement with previous reports,[6,7,9] only a minority of patients (4/12) displayed an HEV-specific CD8+ T-cell response that was narrowly focused, targeting only 1-2 epitopes (Fig. 3A,I). Using tetramer-based enrichment and thus a very sensitive method, we were able to detect HEV-specific CD8+ T cells in 4/9 patients with chronic HEV infection *ex vivo* (Fig. 4A,B). Of note, these HEV-specific CD8+ T cells displayed an activated and predominantly CD127-PD1+ phenotype similar to terminally exhausted CD8+ T cells in chronic HBV and HCV infection.[14,15] Although this phenotype has been described in various chronic disease and cancer settings before, our study is the first to indicate that chronic antigen stimulation could also progress towards T-cell exhaustion under ongoing immunosuppression. Interestingly, exhausted HEV-specific CD8+ T cells were sensitive to immunosuppression and could only be reinvigorated in a substantial number of patients upon reduction of immunosuppression and/or application of ribavirin (Fig. 3G,H), leading to the maintenance of memory-like HEV-specific CD8+ T cells after viral clearance (Fig. 4D). Importantly, the effector function of exhausted CD8+ T cells was reduced but not abolished, as indicated by ongoing expression of GranB and IFNγ (Fig. 3A-D and 4E), and they presumably still exert some degree of viral control. HEV-specific CD8+ T-cell responses declined with increasing duration of persistent HEV infection (Fig. 3H), indicating that long-term chronic HEV infection may lead to a reduced chance of partially reinvigorating functional CD8+ T-cell responses. Thus, prolonged antigenic stimulation may lead to long-lasting epigenetic scars on virus-specific CD8+ T cells, as observed in chronic HCV infection.[16–18] Thus, "standard" treatment of HEV infection (reduction of immunosuppression, ribavirin) as well as future immune-mediated treatment strategies might be the most promising in relatively recent persisting HEV infection, underlining the importance of early diagnosis and treatment.

In a patient who developed chronic HEV infection during abatacept treatment, we could narrowly follow the kinetics of HEV-specific CD8+ T cells and viral clearance upon pausing abatacept. Importantly, this patient displayed a strong increase of HEV-specific CD8+ T cells that preceded a sharp decline in viremia, indicating a direct involvement of HEV-specific CD8+ T cells in viral clearance. This important role of HEV-specific CD8+ T cells was further supported by the longitudinal course of a patient who relapsed after ribavirin but only succeeded in viral clearance with a second course of ribavirin treatment that was associated with the occurrence of HEV-specific CD8+ T cells (Fig. 5F,G). These observations in the human setting are in agreement with recent data from the chicken as well as rhesus macaque HEV infection models that indicated an important role of HEV-specific CD8+ T cells in viral clearance.[3,4] The number of HEV-specific T-cell responses correlated negatively with viral load as well as ALT levels in chronic HEV infection (Fig. S4C,D). This interesting finding could either imply that a high HEV-specific T-cell reactivity contributes to viral control, or that high viral loads contribute to HEV-specific T-cell exhaustion. In addition, they suggest that ALT levels rather indicate non-specific (bystander) inflammation in the absence of HEV-specific immunity and are not a consequence of HEV-specific immunity. Importantly, all our data analyzed the HEV-specific CD8+ T-cell response in peripheral blood. Whether these analyses reflect the CD8+ T-cell response homing to the liver remains to be elucidated by future studies.

Next to T-cell exhaustion, we also found evidence for mutational viral escape in chronic HEV infection, however, this observation was limited to a single epitope in 1 individual (Fig. 6), while the other 4 analyzed patients with persistent infection did not display mutations in the epitopes targeted in the respective patients or HLA-matched epitopes identified in other patients in our study.

In sum, our study led to the identification and fine-mapping of a large set of HEV-specific CD8+ T-cell epitopes that allowed us to study the mechanisms of CD8+ T-cell failure in persistent HEV infection on a single-epitope level. These epitopes will also allow for the study of mechanisms of immunopathology and extrahepatic manifestations in fulminant HEV infection,[19] and they may serve as targets for therapeutic TCR-redirection of T cells against chronic HEV infection.[12] While our understanding of the immunopathology of chronic HEV infection under immunosuppression is still incomplete, the main concept so far implied that immunosuppression prevents T-cell priming. However, our data suggests that an alternative concept may also apply: T-cell responses are primed even in the context of immunosuppression, however, they are dysfunctional, become exhausted and are eventually depleted during ongoing antigen recognition. Importantly, cessation of immunosuppressive medication could restore T-cell responses, while viral escape can contribute to T-cell failure. Our results also indicate that treatments aiming at restoring autologous HEV-specific CD8+ T-cell immunity, *e.g.* by reducing immunosuppression in combination with ribavirin, should be started early in persistent HEV infection. In later stages of chronic HEV infection, more personalized approaches such as the generation and application of TCR-redirected CD8+ T cells might become necessary for viral clearance in patients who fail standard treatment [12]; however,

mutational viral escape should be excluded before this work- and cost-intensive procedure.

Abbreviations

ALT, alanine aminotransferase; CTLA-4, cytotoxic T lymphocyte antigen-4; GranB, granzyme B; IFN, interferon; ORF, open reading frame; PBMCs, peripheral blood mononuclear cells; PD1, programmed cell death 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2; TCF1, T-cell factor 1; TCR, T-cell receptor.

Financial support

The study was partially funded by German Research Foundation (DFG; SFB1160, project number 256073931, to R.T., M.H., C.N.H., Y.T., and K.W.; TRR179, project number 272983813, to T.B.; SFB1328, project number 335447717, to J.S.z.W.; SFB841, project number 80750187, to M.L. and J.S.z.W.) and the German Center for Infection Research (DZIF; TTU Hepatitis, to M.L., S.P., J.S.z.W., and N.F.). M.H. was supported by a Margarete von Wrangell fellowship (State of Baden-Wuerttemberg). D.A.P. was supported by a Welcome Trust Senior Investigator Award (100326/Z/12/Z). The funding bodies had no role in the decision to write or submit the manuscript.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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

Authors' contributions

J.K. planned, performed, and analyzed experiments. S.G. performed HEV UDS sequencing experiments and interpreted sequencing data with the help of N.F. J.H. performed the bioinformatic analysis of the sequencing experiments. S.L.-L. and D.A.P. provided tetramers. F.E. performed four-digit HLA-typing by next-generation sequencing. M.P., D.H., M.L., S.P., J.S.z.W., Y.T., K.W., and T.B. recruited patients and collected clinical data. M.H. and C.N.H. designed the study and contributed to experimental design and planning. J.K., R.T., N.F., M.H., and C.N.H. interpreted data and wrote the manuscript.

Data availability statement

No data is deposited on public data bases. All requests for raw and analyzed data and materials will be reviewed by the corresponding author to verify if the request is subject to any confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical examination and may be subject to patient confidentiality. Any data and materials that can be shared will be released via a material transfer agreement.

Acknowledgments

We thank all patients and healthy donors for participating in the current study.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.05.019.

References

Author names in bold designate shared co-first authorship

[1] EASL clinical practice guidelines on hepatitis E virus infection. J Hepatol 2018;68:1256–1271.
[2] Horvatits T, Schulze Zur Wiesch J, Lutgehetmann M, Lohse AW, Pischke S. The clinical perspective on hepatitis E. Viruses 2019;11.
[3] **Bremer W**, **Blasczyk H**, Yin X, Duron ES, Grakoui A, Feng Z, et al. Resolution of hepatitis E virus infection in CD8+ T cell depleted rhesus macaques. J Hepatol 2021;75:557–564.
[4] Rogers E, Todd SM, Pierson FW, Kenney SP, Heffron CL, Yugo DM, et al. CD8+ lymphocytes but not B lymphocytes are required for protection against chronic hepatitis E virus infection in chickens. J Med Virol 2019;91:1960–1969.
[5] Walker CM. Adaptive immune responses in hepatitis A virus and hepatitis E virus infections. Cold Spring Harb Perspect Med 2018.
[6] Al-Ayoubi J, Behrendt P, Bremer B, Suneetha PV, Gisa A, Rinker F, et al. Hepatitis E virus ORF 1 induces proliferative and functional T-cell responses in patients with ongoing and resolved hepatitis E. Liver Int 2018;38:266–277.
[7] **Brown A**, **Halliday JS**, Swadling L, Madden RG, Bendall R, Hunter JG, et al. Characterization of the specificity, functionality, and durability of host T-cell responses against the full-length hepatitis E virus. Hepatology 2016;64:1934–1950.
[8] Gisa A, Suneetha PV, Behrendt P, Pischke S, Bremer B, Falk CS, et al. Cross-genotype-specific T-cell responses in acute hepatitis E virus (HEV) infection. J Viral Hepat 2016;23:305–315.
[9] Suneetha PV, Pischke S, Schlaphoff V, Grabowski J, Fytili P, Gronert A, et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. Hepatology 2012;55:695–708.
[10] Wu T, Zhang J, Su ZJ, Liu JJ, Wu XL, Lin CX, et al. Specific cellular immune response in hepatitis E patients. Intervirology 2008;51:322–327.
[11] Abravanel F, Barrague H, Dorr G, Saune K, Peron JM, Alric L, et al. Conventional and innate lymphocytes response at the acute phase of HEV infection in transplanted patients. J Infect 2016;72:723–730.
[12] Soon CF, Behrendt P, Todt D, Manns MP, Wedemeyer H, Sallberg Chen M, et al. Defining virus-specific CD8+ TCR repertoires for therapeutic regeneration of T cells against chronic hepatitis E. J Hepatol 2019;71:673–684.
[13] El Costa H, Gouilly J, Abravanel F, Bahraoui E, Peron JM, Kamar N, et al. Effector memory CD8 T cell response elicits hepatitis E virus genotype 3 pathogenesis in the elderly. PLoS Pathog 2021;17:e1009367.
[14] Wieland D, Kemming J, Schuch A, Emmerich F, Knolle P, Neumann-Haefelin C, et al. TCF1+ hepatitis C virus-specific CD8+ T cells are maintained after cessation of chronic antigen stimulation. Nat Commun 2017;8:15050.
[15] Schuch A, Salimi Alizei E, Heim K, Wieland D, Kiraithe MM, Kemming J, et al. Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load. Gut 2019;68:905–915.
[16] **Hensel N**, **Gu Z**, **Sagar**, Wieland D, Jechow K, Kemming J, et al. Memory-like HCV-specific CD8+ T cells retain a molecular scar after cure of chronic HCV infection. Nat Immunol 2021;22:229–239.
[17] Tonnerre P, Wolski D, Subudhi S, Aljabban J, Hoogeveen RC, Damasio M, et al. Differentiation of exhausted CD8+ T cells after termination of chronic antigen stimulation stops short of achieving functional T cell memory. Nat Immunol 2021;22:1030–1041.
[18] Yates KB, Tonnerre P, Martin GE, Gerdemann U, Al Abosy R, Comstock DE, et al. Epigenetic scars of CD8+ T cell exhaustion persist after cure of chronic infection in humans. Nat Immunol 2021;22:1020–1029.
[19] Pischke S, Hartl J, Pas SD, Lohse AW, Jacobs BC, Van der Eijk AA. Hepatitis E virus: infection beyond the liver? J Hepat 2017;66:1082–1095.