Immune responses and immunopathology in acute and chronic viral hepatitis

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Abstract | Hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are responsible for most cases of viral hepatitis. Infection by each type of virus results in a different typical natural disease course and clinical outcome that are determined by virological and immunological factors. HCV tends to establish a chronic persistent infection, whereas HAV does not. HBV is effectively controlled in adults, although it persists for a lifetime after neonatal infection. In this Review, we discuss the similarities and differences in immune responses to and immunopathogenesis of HAV, HBV and HCV infections, which may explain the distinct courses and outcomes of each hepatitis virus infection.

Several human viruses display hepatotropism; that is, they preferentially infect hepatocytes and cause liver inflammation, which is known as viral hepatitis. Most cases of viral hepatitis worldwide are caused by hepatitis A virus (HAV), hepatitis B virus (HBV) or hepatitis C virus (HCV). HCV infection often progresses to chronic persistent infection[1]. Acute HBV infection spontaneously resolves in more than 90% of infected adults, although HBV can sometimes result in chronic persistent infection, particularly when neonates are infected through vertical transmission[2]. As a result, approximately 170 million and 350 million people worldwide are chronically infected with HCV and HBV, respectively, and infected individuals are at an increased risk of liver cirrhosis and hepatocellular carcinoma[3]. HAV infection often causes severe liver injury in adults, whereas it results in an asymptomatic subclinical infection in children. However, HAV infection is effectively controlled by the host and does not progress to chronic infection[4]. Currently, prophylactic vaccines are available against HAV and HBV; therefore, *de novo* infection by HAV and HBV is significantly decreasing in developed countries. By contrast, a prophylactic vaccine is not yet available against HCV. The basic characteristics of HAV, HBV and HCV are summarized in TABLE 1. In addition to these three hepatitis viruses, hepatitis E virus (HEV), which is a positive-strand non-enveloped RNA virus, is an increasing public-health concern[5]. Whereas HEV usually causes acute self-limiting infection after faecal–oral transmission, chronic infection can occur in immunocompromised patients[5]. Currently, however, immune responses to HEV are not well understood and thus are not discussed in this Review.

HAV, HBV and HCV differ in terms of their virological characteristics. HAV and HCV are positive-strand RNA viruses that belong to the Picornaviridae and Flaviviridae families, respectively[1]. HBV is a partially double-stranded DNA virus, and reverse transcriptase is required for its replication. In infected cells, HBV exists as a form of episomal DNA, known as covalently closed circular DNA (cccDNA), which is a viral transcriptional template[2] and is an important hurdle in terms of virus eradication. Whereas chronic HCV infection can be cured with direct-acting antivirals (DAAs), it is difficult to cure chronic HBV infection with nucleoside and nucleotide analogue antivirals because cccDNA cannot be eliminated[3].

HAV, HBV and HCV infections follow typical courses and outcomes depending on the infecting virus (FIG. 1). The distinctive courses and outcomes of each hepatitis virus infection are determined by both the virological characteristics and the immune responses that are elicited by each hepatitis virus. This Review discusses and compares innate and adaptive immune responses to HAV, HBV and HCV. Furthermore, the mechanisms of immune-mediated liver injury are described. In this Review, HBV infection is mainly discussed in the setting of infection in adults. Perinatal HBV infection is briefly described in BOX 1.

Type I and type III interferon responses

Type I interferons (IFNα and IFNβ proteins) and type III IFNs (IFNλ proteins) are major components of the antiviral innate immune system. Type I and type III IFNs bind to the IFNα/β receptor (IFNAR1–IFNAR2) and the IFNλ receptor (IFNLR1–IL10R2), respectively[6].

Covalently closed circular DNA
(cccDNA). The replicative form of hepatitis B virus (HBV) DNA, which exists in a circular form as a plasmid-like episome in the host cell nucleus. It is an essential component of the HBV replication cycle and responsible for virus persistence.

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Table 1 | **Virological and clinical features of HAV, HBV and HCV**

Feature	HAV	HBV	HCV
Virology			
Genome	Positive-strand RNA	Partially double-stranded DNA	Positive-strand RNA
Virus structure	28 nm; non-enveloped nucleocapsid; 'enveloped' form has recently been discovered[41]	42 nm; enveloped nucleocapsid	50 nm; enveloped nucleocapsid
Classification	Picornaviridae family; Picornavirus	Hepadnaviridae family; Orthohepadnavirus	Flaviviridae family; Hepacivirus
Genotype	Three major genotypes; six subtypes	Eight genotypes	Six major genotypes; more than 50 subtypes
Mutation rate	Unknown	Low	High
Virus half-life	Unknown	2–3 days	3 hours
Virion production	Unknown	10^{10}–10^{12} virions per day	10^{12} virions per day
Epidemiology			
Worldwide prevalence	>1.5 million people infected per year	350 million people chronically infected	170 million people chronically infected
Transmission	Enteral, faecal–oral transmission	Mostly vertical (or perinatal) transmission in endemic areas; horizontal transmission by intravenous drug use or other parenteral routes; sexual transmission	Mostly horizontal transmission by intravenous drug use or other parenteral routes
Natural course of infection			
Incubation period	10–50 days (average 25–30 days)	50–180 days (average 60–90 days)	40–120 days
Manifestations or symptoms	Mostly subclinical in children; frequently symptomatic in adults	Frequently symptomatic in adults	Mostly subclinical
Persistent infection	Self-limited; does not develop into chronic infection	Persists in >90% of cases after vertical transmission; persists in <10% of cases after horizontal transmission	Persists in 60% to ~80% of cases after acute infection
Treatment of infection			
IFNα-based treatment	—	Pegylated IFNα; HBs seroconversion* in a minority of patients; slow reduction in viraemia	Pegylated IFNα plus ribavirin; virus clearance in 45–80% of patients depending on HCV genotype; fast reduction in viraemia
IFNα-free treatment	Supportive care	Nucleoside or nucleotide analogues; no elimination of HBV cccDNA; fast reduction in viraemia	DAAs targeting NS3, NS5A and NS5B; high therapeutic efficacy (>90%); fast reduction in viraemia

cccDNA, covalently closed circular DNA; DAAs, direct-acting antivirals; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; IFNα, interferon-α. *HBs seroconversion is the loss of hepatitis B surface antigen (HBsAg) and the development of HBsAg-specific antibodies.

Direct-acting antivirals
(DAAs). Therapeutic drugs for hepatitis C virus (HCV) infection that directly interfere with the function of virus proteins at various stages of the HCV replication cycle. DAAs targeting NS3–NS4A protease, NS5A and NS5B polymerase have been approved for clinical use. DAAs have greatly improved therapeutic efficacies and safety profiles compared with pegylated IFNα–ribavirin combination therapy.

Nucleoside and nucleotide analogue antivirals
Derivatives of standard nucleosides and nucleotides that are incorporated into the DNA or RNA during virus replication, leading to chain termination and, thereby, inhibition of virus replication. Those used for the treatment of hepatitis B virus (HBV) infection inhibit the activity of HBV polymerase, thus suppressing virus replication.

IFN-stimulated genes
(ISGs). Genes that are transcriptionally upregulated by interferons (IFNs) through binding of the transcription factor ISGF3 to IFN-stimulated response elements (ISREs). The protein products of these genes have diverse functions in cells, including antiviral functions.

Both type I and type III IFNs induce the expression of IFN-stimulated genes (ISGs) mediated by an IFN-stimulated gene factor 3 (ISGF3) complex formed by phosphorylated signal transducer and activator of transcription 1 (STAT1), phosphorylated STAT2 and IFN regulatory factor 9 (IRF9)[7] (FIG. 2).

Early studies of IFN responses in hepatitis virus infection examined the expression profiles of ISGs in the livers of virus-infected chimpanzees. HCV infection increased the expression of many ISGs[8], whereas HAV infection minimally induced ISG expression[9] (FIG. 1). HBV infection did not induce ISG expression[10]; thus, HBV was considered to be a 'stealth virus' that is not recognized by the innate immune system[11].

HCV infection. Cytosolic HCV RNA is sensed by retinoic acid-inducible gene I (RIG-I; also known as DDX58) and melanoma differentiation-associated protein 5 (MDA5; also known as IFIH1) in a sequential manner (FIG. 2a). The RIG-I-mediated IFN response dominates at early stages of infection, whereas the MDA5-mediated IFN response occurs later[12]. Protein kinase R (PKR; also known as

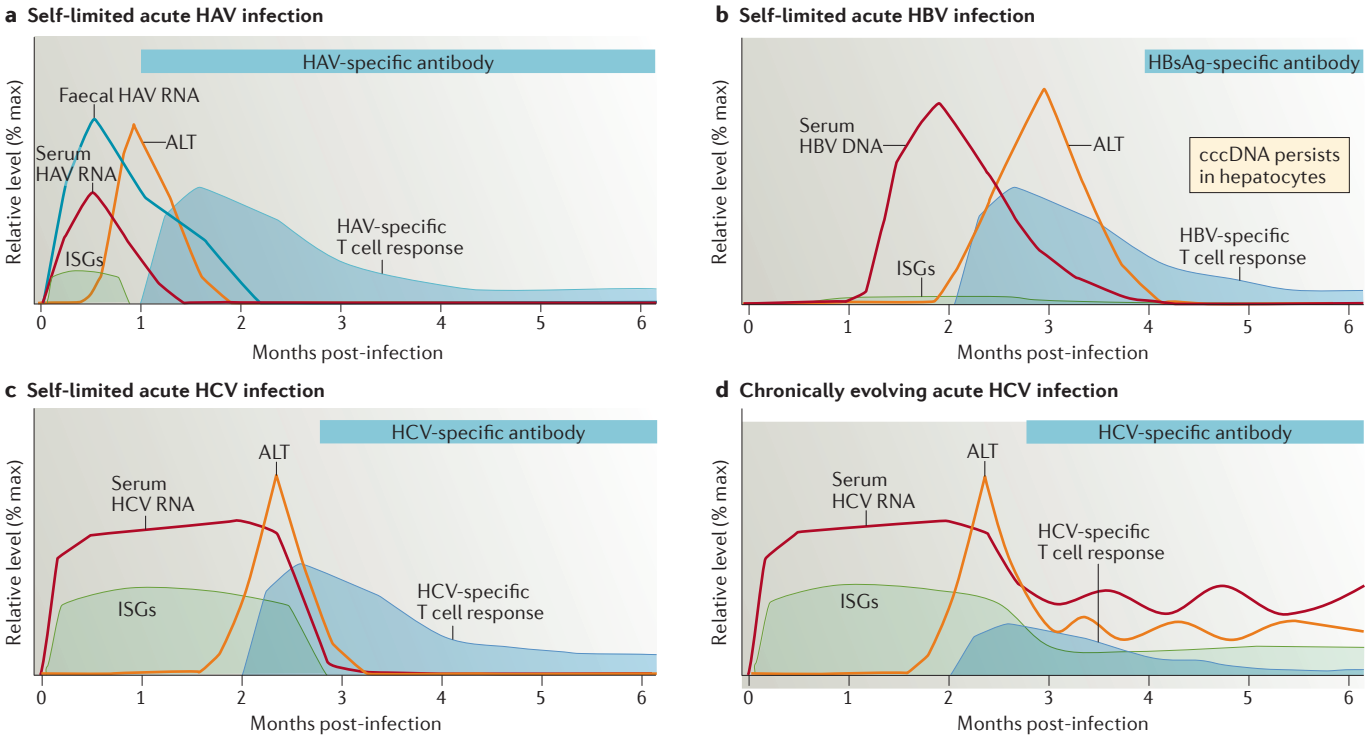

Figure 1 | **Typical time courses of acute hepatitis virus infections.** The graphs show typical time courses of hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infections during the first 6 months in chimpanzee infection models. Whereas acute HAV (part **a**) and HBV (part **b**) infections are well controlled by hosts, acute HCV infection can result in divergent outcomes: either self-limited infection (part **c**) or progression to chronic infection (part **d**). A rapid increase in serum HAV RNA (part **a**) or HCV RNA (parts **c**,**d**) levels occurs immediately after infection. By contrast, the increase in serum HBV DNA levels is delayed after infection depending on the HBV infection dose[106]. A representative case of HBV infection is shown[176] (part **b**). The hepatic expression of IFN-stimulated genes (ISGs) is increased during acute HCV infection (parts **c**,**d**), whereas it is only minimally increased in acute HAV infection (part **a**) and is not increased in acute HBV infection (part **b**). In acute HCV infection, virus-specific T cell responses are induced in a delayed manner despite the early increase in virus loads (parts **c**,**d**). In acute HAV (part **a**) and HBV (part **b**) infections, virus-specific T cell responses are not considerably delayed in relation to the kinetics of the increase in virus loads. In all types of infection, T cell responses coincide with liver injury (as indicated by increased serum levels of alanine aminotransferase (ALT)) and virus control, even if the virus is not completely cleared. Virus-neutralizing antibodies develop during self-limited acute HAV and HBV infections and confer life-long protective immunity to infection. Although a role for virus-specific antibodies in the control of acute HCV infection has recently been reported, it remains to be clearly elucidated.

EIF2AK2) also participates in early sensing of HCV. After binding to HCV dsRNA, PKR interacts with mitochondrial antiviral signalling protein (MAVS) and induces ISGs[13]. Toll-like receptor 3 (TLR3) also induces IFN production and ISG expression through sensing HCV dsRNA in endosomes[14]. Once type I and type III IFNs are produced, they can suppress HCV replication by the antiviral action of ISGs[15]. Therefore, pegylated IFNα (peg-IFNα) has been used for the treatment of HCV infection.

HCV interferes with the induction of IFNs[7]. The HCV NS3–NS4A serine protease cleaves MAVS independently of its subcellular location, such as in the mitochondria, in mitochondria-associated endoplasmic reticulum membranes (MAMs)[16] and in peroxisomes[17] (FIG. 2a). MAVS cleavage by HCV NS3–NS4A was confirmed in HCV-infected liver tissue[18]. Furthermore, HCV NS3–NS4A can cleave Toll/IL-1 receptor domain-containing adaptor inducing IFNβ (TRIF; also known as TICAM1), which is a downstream mediator of TLR3-induced signal transduction[19].

Pegylated IFNα
(peg-IFNα). Recombinant interferon-α (IFNα) modified by the addition of polyethylene glycol. This modification increases the half-life of recombinant IFNα in the circulation and improves antiviral efficacy compared with the native form.

Although HCV interferes to some extent with the induction of IFNs, type I and type III IFNs are still produced during HCV infection. ISGs are continuously upregulated in the HCV-infected livers of chimpanzees[8], and ISG mRNA is simultaneously detected with HCV RNA in the hepatocytes of patients who are infected with HCV[20]. In HCV cell culture models, the production of IFNs, particularly IFNλs rather than IFNβ, in response to HCV infection has been demonstrated[21,22]. A recent study using laser-capture micro-dissection showed that IFNλs are induced only in HCV-infected cells and not in neighbouring uninfected cells[23]. In addition, plasmacytoid dendritic cells (pDCs) also produce type I IFNs during HCV infection through TLR7-mediated recognition of HCV RNA loaded in exosomes that are released from infected hepatocytes[24] (FIG. 2a).

Chronic HCV infection. As described above, IFNs and ISGs are expressed during HCV infection despite the interference mechanisms of the virus. As long as

Box 1 | **Lifelong course of perinatal HBV infection**

In endemic areas, hepatitis B virus (HBV) is mostly transmitted from chronically infected mothers to neonates, although this transmission can be prevented by both passive and active immunization. Perinatal HBV infection results in chronic infection in more than 90% of exposed individuals[158]. Lifelong mortality that is caused by liver cirrhosis or hepatocellular carcinoma reaches 40% in men and 15% in women with perinatal HBV infection[158].

Chronic lifelong HBV infection owing to perinatal HBV exposure has several phases. In most infected children, liver inflammation is minimal despite high rates of HBV replication; this is known as the 'immune-tolerant' phase[105,158]. This phase can last for decades and is followed by the 'immune-clearance' (or 'immunoactive') phase, which is characterized by liver inflammation and decreased HBV replication[105,158]. The immune-clearance phase is followed by the 'low-replicative' phase, with minimal liver inflammation. However, 20–30% of patients undergo a 'reactivation' phase, which is characterized by increased HBV replication and liver inflammation. Patients in this phase have an increased risk of liver cirrhosis and hepatocellular carcinoma.

The underlying mechanism to explain why perinatal transmission of HBV often leads to chronic persistent infection has not been elucidated. Children in the immune-tolerant phase are considered to have impaired immune functions, which prevents them from mounting an effective antiviral response[159]. In particular, impaired interleukin-21 (IL-21) production has been implicated in the defective antiviral immune responses in this phase[160]. By contrast, a recent investigation of the immune profile of children and young adults with immune-tolerant chronic HBV infection showed that they had preserved T cell function against HBV and had no T cell tolerance[161]. Further studies with a larger scale and a longitudinal design are required to better understand the mechanisms of immune-tolerance and immune-clearance in lifelong HBV infection.

HCV persists within the host, type I and type III IFNs are produced, and many ISGs are upregulated during chronic HCV infection[8]. However, phosphorylated STAT1 is scarcely detected in chronically HCV-infected livers[25]. Instead, a different transcription factor complex known as unphosphorylated ISGF3 (U-ISGF3) — which is formed by high protein levels of STAT1, STAT2 and IRF9 without tyrosine phosphorylation of the STATs[26] — has a role in the induction of a set of ISGs[25]. Moreover, U-ISGF3 maintains the sustained expression of these particular ISGs for a prolonged time in HCV-infected cells (FIG. 2b), thus conferring extended antiviral effects[25].

However, the sustained expression of ISGs has an adverse effect on spontaneous virus clearance and on IFNα-based treatment. Patients infected with HCV who have high levels of ISGs in their livers at baseline have worse rates of spontaneous virus clearance[27] and respond poorly to combination therapy with peg-IFNα and ribavirin[28–30]. In the setting of IFNα-based therapy, ubiquitin-specific peptidase 18 (USP18) has been emphasized as a negative regulator that is responsible for unresponsiveness to IFNα[31]. *USP18* is itself an ISG; however, USP18 interacts with IFNAR2 and inhibits downstream signals that are induced by IFNα[32]. A recent study found that prolonged exposure to endogenously produced IFNλs during HCV infection induces the sustained upregulation of *ISG15* by U-ISGF3 (REF. 25) (FIG. 2b). ISG15 stabilizes the USP18 protein[33] and causes the unresponsiveness to exogenous IFNα treatment[25] (FIG. 2c). ISG15 is one of the most abundantly expressed ISGs in HCV-infected livers, and this novel function of ISG15 helps to explain why high baseline levels of ISGs in HCV-infected livers are associated with a poor response to IFNα-based therapy. The response to IFNα-based therapy is also strongly influenced by the recently identified *IFNL4* genotype[34,35]. This issue is further described in BOX 2.

HAV infection. The cytosolic RNA of picornaviruses such as HAV is sensed by MDA5 (REF. 36). However, in contrast to HCV, HAV minimally stimulates IFN responses in the infected liver[9]. In chimpanzee studies, although the amount of viral RNA was much greater in HAV-infected livers than in HCV-infected livers, the expression of ISGs was barely induced in HAV-infected livers, whereas it was strongly increased in HCV-infected livers[9]. These findings indicate that HAV has evolved more effective strategies than HCV to inhibit the induction of IFNs in infected cells. Indeed, HAV proteins and their precursors block the IFN response in several ways. For example, an intermediate product of HAV polyprotein processing, 3ABC, targets MAVS for proteolysis[37], and another precursor, 3CD, cleaves TRIF (downstream of TLR3)[38]. HAV 3C protease cleaves NF-κB essential modulator (NEMO; also known as IKBKG), thereby attenuating nuclear factor-κB (NF-κB) activation downstream of both MAVS and TLR3 (REF. 39). In HAV infection, the high level of viral replication may lead to a higher level of expression of viral proteins than in HCV infection, which thus blocks the induction of IFNs more efficiently[4]. The minimal IFN response in the HAV-infected liver can also be explained by the disappearance of pDCs[40]. In HAV infection, pDCs produce a substantial amount of IFNα by sensing 'enveloped' HAV[40] (that is, virus particles cloaked in host-derived membranes[41]). However, pDCs disappear from the HAV-infected liver after peak viraemia[40]. Although HAV infection induces minimal IFN responses, it is noteworthy that the expression of CXC-chemokine ligand 10 (CXCL10), which is a representative IFN-inducible chemokine, is increased in the liver and blood during acute HAV infection[9,42].

HBV infection. As described above, HBV is sometimes known as a stealth virus as it does not seem to be sensed by the host[11]. However, some studies have shown that HBV infection can be sensed and thus can induce an IFN response: a recent study showed that the 5′-ε region of HBV (genotypes A, B and C) pre-genomic RNA (pgRNA) is recognized by RIG-I, resulting in the induction of IFNλs[43]; another study reported that MDA5, but not RIG-I, senses HBV (genotype D)[44].

Similarly to HCV and HAV, HBV uses several strategies to interfere with the IFN response. HBV polymerase blocks TLR3- and RIG-I-induced IRF activation by inhibiting TANK-binding kinase 1 (TBK1)–IκB kinase-ε (IKKε)[45]. HBV polymerase also inhibits stimulator of interferon genes (STING)-stimulated IRF3 activation by disrupting the Lys63-linked ubiquitylation of STING, a protein that induces IFNs in response to the recognition of foreign DNA[46]. Furthermore, HBV polymerase inhibits IFNα-induced nuclear translocation of STAT1, thus interfering with the induction of ISGs[47]. In addition, HBx, a multifunctional regulatory protein of HBV, downregulates the expression of MAVS[48].

Ribavirin
A guanosine analogue that has antiviral activity against a broad range of RNA viruses by interfering with RNA metabolism. It has been used in combination with pegylated interferon-α (peg-IFNα) for the treatment of hepatitis C virus (HCV) infection.

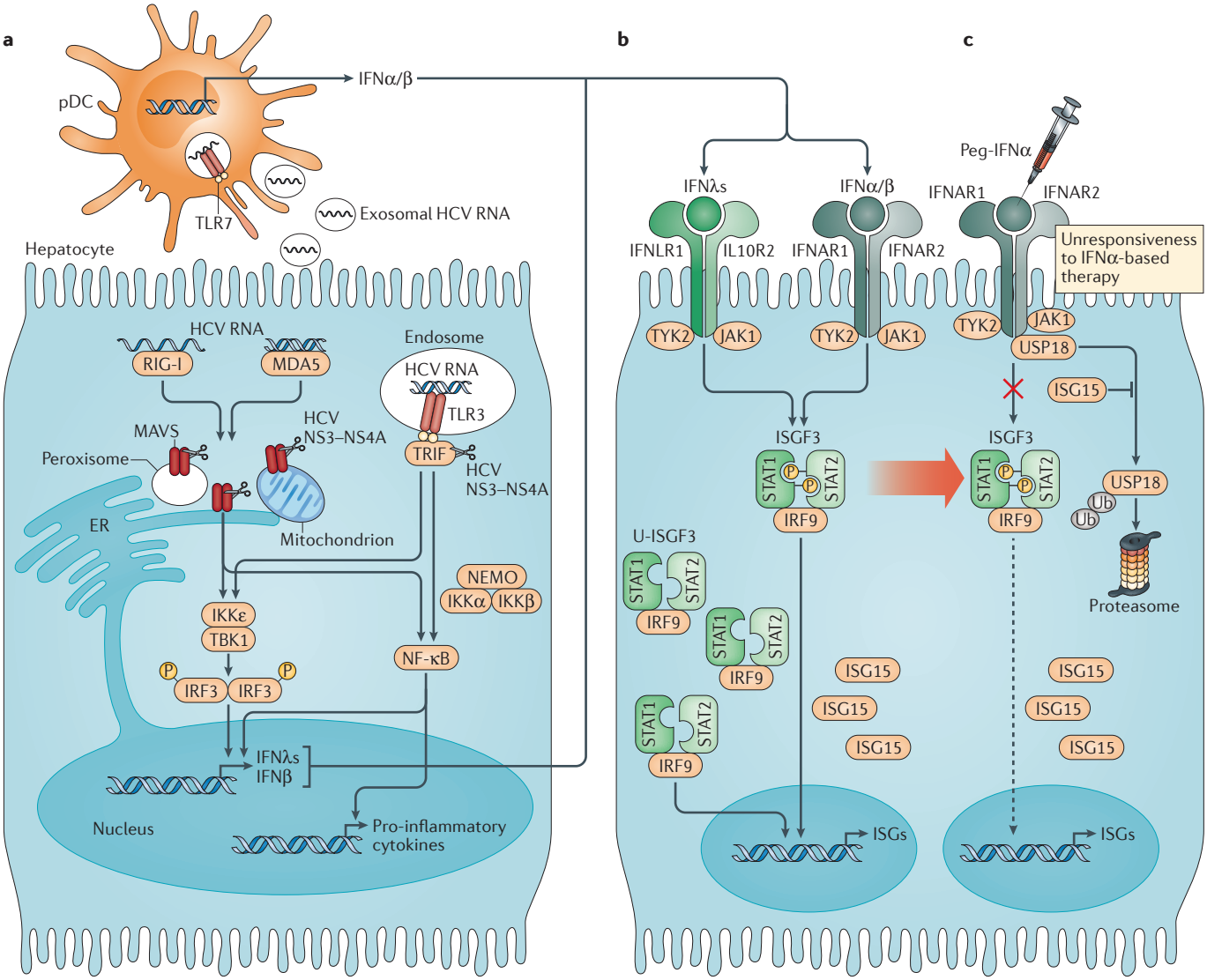

Figure 2 | **Type I and type III IFN responses in HCV infection. a** | Hepatitis C virus (HCV) RNA is sensed by retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5) and Toll-like receptor 3 (TLR3) in infected hepatocytes. Downstream signalling results in the induction of interferon-λ proteins (IFNλs), IFNβ and other pro-inflammatory cytokines such as tumour necrosis factor and CXC-chemokine ligand 10. Plasmacytoid dendritic cells (pDCs) recognize exosomes loaded with HCV RNA, which are released from infected hepatocytes, and respond by producing type I IFNs. HCV NS3–NS4A protease (represented by scissors in the figure) cleaves mitochondrial antiviral signalling protein (MAVS) and Toll/IL-1 receptor domain-containing adaptor inducing IFNβ (TRIF) as a mechanism for interfering with the IFN response. However, IFNs are still produced, and IFN-stimulated genes (ISGs) are induced in HCV-infected hepatocytes. **b** | When type I and type III IFNs bind to their receptors, IFN-stimulated gene factor 3 (ISGF3) — consisting of phosphorylated signal transducer and activator of transcription 1 (STAT1), phosphorylated STAT2 and IFN regulatory factor 9 (IRF9) — induces the expression of ISGs. At chronic stages, the expression of ISGs is maintained by unphosphorylated ISGF3 (U-ISGF3) that is formed by high levels of STAT1, STAT2 and IRF9 in the absence of tyrosine phosphorylation of the STATs. **c** | ISG15 is abundantly expressed in HCV-infected cells. ISG15 stabilizes the ubiquitin-specific peptidase 18 (USP18) protein, and USP18 blocks signals through the IFNα/β receptor. As a consequence, HCV-infected cells become unresponsive to exogenous IFNα (including pegylated IFNα (peg-IFNα)) treatment. ER, endoplasmic reticulum; IKKε, IκB kinase-ε; IRF, IFN regulatory factor; JAK1, Janus kinase 1; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; TBK1, TANK-binding kinase 1; TYK2, tyrosine kinase 2; Ub, ubiquitin.

In HBV infection, the very low level of IFN production and induction of ISGs is determined by a balance between IFN induction and interference mechanisms. In this respect, a slight induction of human ISGs in the livers of HBV-infected chimeric mice harbouring human hepatocytes should be noted[49]. This finding indicates that HBV has very strong interference mechanisms for the IFN response compared with the other hepatitis viruses.

Box 2 | **Importance of *IFNL* genotypes in HCV infection**

For infection with hepatitis C virus (HCV) genotype 1, which is the most common genotype in Western countries, 50% of patients fail to achieve a sustained virological response to combination therapy with pegylated interferon-α (peg-IFNα) and ribavirin. In 2009, several groups reported that single nucleotide polymorphisms (SNPs) located near the *IFNL3* locus (encoding IFNλ3 (also known as IL-28B)) are strongly associated with the response to peg-IFNα–ribavirin therapy[162–164] and with the spontaneous clearance of HCV infection[165]. Patients with the *rs12979860*-T allele (C/T or T/T) respond poorly to peg-IFNα–ribavirin treatment, whereas patients with two copies of the *rs12979860*-C allele (C/C) respond well to this treatment.

In 2013, the *IFNL4* gene (encoding IFNλ4) was discovered near the *IFNL3* locus[34]. Interestingly, IFNλ4 expression is influenced by a germline dinucleotide frameshift variant that is located in exon 1 of the *IFNL4* gene (*rs368234815*)[34]. The *rs368234815*-ΔG allele generates the full-length IFNλ4 protein, whereas the *rs368234815*-TT allele does not generate IFNλ4 because of a premature stop codon[34]. The *rs368234815*-ΔG allele in the *IFNL4* locus is strongly associated with the previously known *rs12979860*-T allele of the *IFNL3* locus by linkage disequilibrium. Patients with chronic HCV infection with the *rs368234815*-ΔG allele (ΔG/TT or ΔG/ΔG) that encodes the functional IFNλ4 protein have high baseline IFN-stimulated gene (ISG) levels in the liver[27] but respond poorly to peg-IFNα–ribavirin treatment[34,35]. A recent study also showed that *IFNL4*-ΔG/TT is the primary *IFNL* gene polymorphism that determines the treatment response to peg-IFNα–ribavirin[35].

A functionally defective form of the IFNλ4 protein is associated with a weaker induction of ISGs in the livers of patients with chronic HCV infection, which indicates that IFNλ4 is the main driver of hepatic ISG induction in chronic HCV infection[27]. Indeed, the IFNλ4 protein activates the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway by binding to the IFNλ receptor[166] and induces the expression of ISGs[167]. However, it remains to be elucidated why patients with the *rs368234815*-ΔG allele (ΔG/TT or ΔG/ΔG) encoding the IFNλ4 protein respond poorly to peg-IFNα–ribavirin treatment.

Natural killer cells

Natural killer (NK) cells are major cellular components of the antiviral innate immune system, and they exert effector functions through cytotoxicity and cytokine production under the regulation of the balance between inhibitory signals and activating signals. In HCV infection, the importance of NK cells was suggested more than 10 years ago by a genetic study of killer-cell immunoglobulin-like receptors (KIRs) and HLA molecules in HCV-exposed individuals[50]. In this study, spontaneous HCV clearance was significantly associated with the *KIR2DL3*/*HLA-C1* compound genotype[50], which is known to result in a lower threshold for NK cell activation[51]. A potential antiviral role for NK cells has also been emphasized in cases of subclinical HCV exposure, such as individuals who injected drugs who were highly exposed to HCV but who were not infected by the virus[52,53] or HCV-exposed healthcare workers who did not develop acute infection without seroconversion[54]. In these cases, protected individuals had an increased frequency of NK cells, as well as increased IFNγ production and cytotoxicity.

In co-culture experiments, it was shown that NK cells inhibit HCV replication by secreting IFNγ[55,56]. In particular, NKp46^{high} NK cells efficiently suppressed HCV replication[57,58]. However, the contribution of NK cells to HCV control *in vivo* during acute HCV infection has not yet been clearly defined.

NK cells undergo functional changes during HCV infection. In acute HCV infection, NK cells have an activated phenotype (increased expression of NKG2D (also known as KLRK1)) and increased effector functions (increased IFNγ production and cytotoxicity), irrespective of the outcome of the infection[59,60]. If the infection progresses to a chronic infection, NK cells are functionally polarized towards increased cytotoxicity and decreased IFNγ production by chronic exposure to type I IFNs[61]. Chronic exposure to type I IFNs increases STAT1 expression in NK cells, which results in STAT1 dominance over STAT4 in intracellular signalling pathways[62,63]. As a consequence, NK cells upregulate expression of the apoptosis-inducing ligand TRAIL (also known as TNFSF10) and have increased cytotoxicity, whereas they have decreased IFNγ production[62,63], which is an important effector function of NK cells for HCV control[56].

NK cells are also functionally changed during HBV infection. In acute HBV infection, the IFNγ-producing capacity of NK cells is transiently reduced, particularly when interleukin-10 (IL-10) is produced[64]. The IFNγ-producing capacity of NK cells remains at a reduced level if the infection progresses to a chronic infection, although TRAIL expression is increased and the cytotoxic activity of NK cells is not impaired during chronic HBV infection[65,66]. This functional alteration of NK cells in response to HBV infection is caused by immunosuppressive cytokines, such as IL-10 and transforming growth factor-β (TGFβ), which are produced by various immune cells[66,67], rather than by type I IFNs as for HCV infection.

In summary, NK cells have a functional dichotomy (decreased IFNγ production and increased or maintained cytotoxicity) in both HBV infection and chronic HCV infection. Considering a cytotoxicity-independent antiviral function of IFNγ[68,69], it has been suggested that a reduced capacity of IFNγ production by NK cells is related to the chronic persistence of HCV and HBV[70]. Meanwhile, increased or maintained cytotoxic activity of NK cells might contribute to liver injury in chronic HCV infection and HBV infection. The functions and phenotype of NK cells have not been characterized in detail in HAV infection.

Neutralizing antibodies

Virus-specific antibodies develop in all cases of hepatitis virus infection, but the roles of these antibodies differ depending on the virus. HAV-specific antibodies with virus-neutralizing activity develop during HAV infection and confer lifelong protective immunity to hosts that recover from HAV infection[4]. HAV-specific neutralizing antibodies are also induced by immunization with inactivated virus-based vaccines[71].

In HBV infection, antibodies that are specific for hepatitis B surface antigen (HBsAg) develop during the course of spontaneously resolving infection. HBsAg-specific antibodies have virus-neutralizing activity and control HBV infection, even with the persistence of cccDNA, thus conferring protective immunity to the hosts[2]. HBsAg-specific antibodies are also induced by immunization with recombinant HBsAg.

In HCV infection, HCV-specific antibodies are not long-lasting even after spontaneous clearance of the virus[72], and the roles of these antibodies in the control

of infection and in protective immunity have not yet been clearly elucidated. In principle, antibodies that are specific for E1 and E2 envelope proteins have virus-neutralizing activity. However, HCV E1- or E2-specific antibodies tend to easily lose their neutralizing activity as a result of virus escape mutations[73]. In addition, cell-to-cell direct transmission of HCV can help the virus to escape detection by neutralizing antibodies[74]. In fact, early studies reported that HCV-specific antibodies have a limited role in the control of infection, as neutralizing antibodies were often detected in patients or chimpanzees with chronic persistent HCV infection rather than in hosts with spontaneous resolution[75,76]. However, these studies assessed neutralizing antibodies using only a limited set of HCV pseudo-particles. In contrast to these early studies, recent studies have reported evidence that supports a role for HCV-specific antibodies in the control of infection[77]. Cross-reactive neutralizing antibodies were induced during early acute infection in patients with spontaneous recovery, whereas neutralizing antibodies developed in a delayed manner in patients with chronic evolution of HCV infection[78], and this was particularly apparent in a study that used an HCV pseudo-particle library[79]. Interestingly, the appearance of neutralizing antibodies was observed in a patient with spontaneous clearance of chronic HCV infection, which rarely occurs[80]. Recently, a crystal structure of E2 was reported[81], and broadly neutralizing antibodies with protective or therapeutic activity have been developed[82,83]. However, the role of HCV-specific antibodies in the natural control of HCV infection must be further clarified. Furthermore, it remains to be determined how broadly neutralizing antibodies can be induced, which would provide valuable information for the development of a prophylactic HCV vaccine.

T cell responses

In acute HCV and HBV infections, T cells have a crucial role in determining spontaneous resolution versus virus persistence. This role is clearly supported by chimpanzee studies showing that *in vivo* depletion of either $CD4^+$ or $CD8^+$ T cells hampers HCV[84,85] and HBV[86] clearance and clinical recovery. In particular, robust and multiple epitope-specific $CD8^+$ T cell responses, which are helped by $CD4^+$ T cells, are necessary for the spontaneous resolution of acute HCV or HBV infection. T cell responses in acute HAV infection have been recently studied, but detailed information is still lacking. If HCV or HBV infection progresses to a chronic persistent infection, virus-specific T cells are exhausted and functionally impaired.

Acute HCV infection. In acute HCV infection, virus-specific T cell responses are remarkably delayed despite the early increase in HCV titres and the early induction of type I and type III IFN responses (FIG. 1). HCV-specific T cells cannot be detected in the blood and liver until 8–12 weeks after infection[87,88], which is caused by a primary delay in the induction of HCV-specific T cells[88]. However, the appearance of HCV-specific T cells and IFNγ expression in the liver coincide with the decline of virus titres by greater than two log regardless of the outcome of the acute HCV infection[88,89] (FIG. 1). The mechanism underlying the delayed induction of T cells remains unknown.

Nevertheless, virus-specific $CD8^+$ T cells have a crucial role in the outcome of acute HCV infection, as supported by immunogenetic studies showing that HLA-B27, HLA-B57 and HLA-A3 allotypes are associated with spontaneous HCV clearance (reviewed in REF. 90). In fact, a vigorous $CD8^+$ T cell response — robust IFNγ production by HCV-specific $CD8^+$ T cells — is a strong immunological correlate of the spontaneous resolution of acute HCV infection (reviewed in REF. 90). Whereas robust HCV-specific $CD8^+$ T cell responses are induced in patients or chimpanzees with a self-limited HCV infection, weak $CD8^+$ T cell responses are observed in hosts who have a chronically evolving infection[87,91–93] (FIG. 3). The early appearance of HCV-specific $CD8^+$ T cells that express the memory precursor marker CD127 during the acute phase of infection corresponds to increased production of IFNγ and tumour necrosis factor (TNF) by $CD8^+$ T cells in chimpanzees that subsequently achieved a self-limited HCV infection[93].

However, the antiviral action of $CD8^+$ T cells can be abolished by virus escape mutations inside or in the flanking regions of $CD8^+$ T cell epitopes[94], although escape mutations may diminish the replication fitness of the virus. Therefore, the breadth of the HCV-specific $CD8^+$ T cell response is another crucial factor in determining the outcome of acute HCV infection. $CD8^+$ T cell responses specific for multiple epitopes contribute to virus eradication by overcoming the emergence of virus escape mutants, whereas narrow $CD8^+$ T cell responses are associated with virus persistence[90,91].

$CD4^+$ T cells also have an important role in the spontaneous resolution of acute HCV infection. Strong and broad HCV-specific $CD4^+$ T cell responses are observed in most acutely infected patients irrespective of infection outcome; however, $CD4^+$ T cell proliferation is decreased and IL-2 production is diminished in patients with virus persistence[95,96]. The loss of $CD4^+$ T cell help leads to diminished HCV-specific $CD8^+$ T cell responses[87,92] (FIG. 3). Thus, $CD8^+$ T cells mediate the spontaneous resolution of acute HCV infection only when $CD4^+$ T cell help is simultaneously maintained.

A recent study reported that HCV-specific T follicular helper cells (T_{FH} cells) are detected in the blood of patients with acute HCV infection and that expression of inducible T cell co-stimulator (ICOS) by these cells correlates with the production of HCV-specific antibodies[97]. T_{FH} cells are detectable in the liver, but not in the blood, of patients with chronic HCV infection[97].

During acute HCV infection, the frequency of $CD4^+CD25^+FOXP3^+$ regulatory T cells (T_{reg} cells) in the blood tends to transiently increase[98–100], although this occurs irrespective of the outcome of the infection[99]. Moreover, an imbalance between T_{reg} cells and T_H17 cells was demonstrated in acute HCV infection. In particular, an increased frequency of galectin 9-expressing T_{reg} cells and a lower frequency of IL-21-producing

Virus escape mutations
Mutations generating viral protein products that can no longer be recognized by virus-specific antibodies or T cells. Viruses with a high rate of mutation, such as hepatitis C virus (HCV) and HIV, rely on escape mutations as a mechanism of immune evasion.

T follicular helper cells
(T_{FH} cells). A distinct subset of $CD4^+$ T cells found in B cell follicles of secondary lymphoid organs. ICOS (inducible T cell co-stimulator)-dependent T_{FH} cells have a crucial role in the selection and survival of B cells that differentiate to plasma cells or memory B cells.

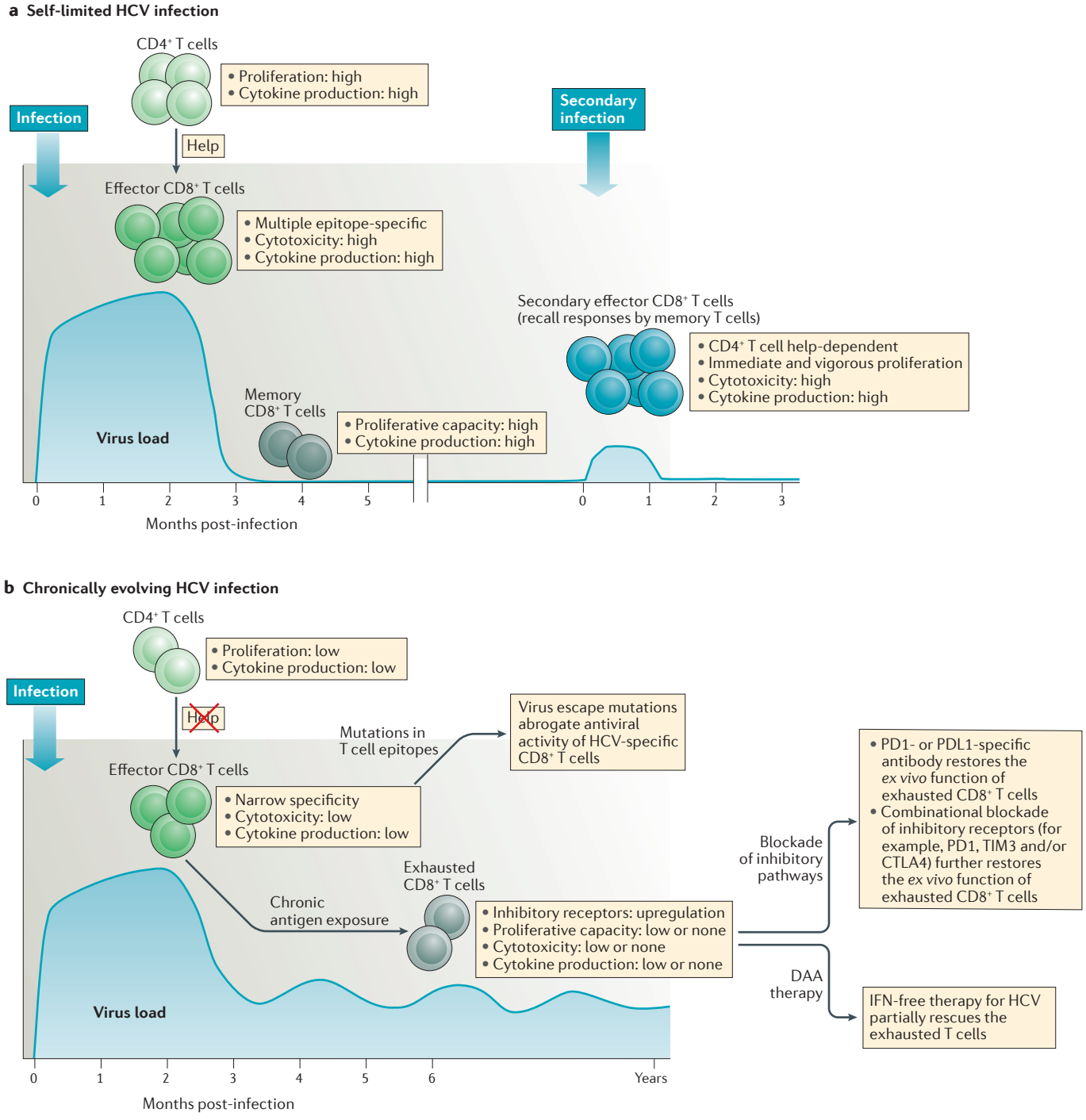

Figure 3 | **T cell responses in acute and chronic HCV infection. a** | Self-limited hepatitis C virus (HCV) infection is associated with strong, broad and polyfunctional CD8$^+$ T cell responses. Help from functional CD4$^+$ T cells during acute infection is essential for the induction and maintenance of effector and memory CD8$^+$ T cells. After the spontaneous resolution of infection, HCV-specific memory CD8$^+$ T cells persist for decades and can respond immediately after secondary infection[174,177]. The CD8$^+$ T cell recall response, which depends on CD4$^+$ T cell help, results in lower levels of viraemia and more rapid virus clearance compared with primary infection[84,85]. **b** | Weak and narrow CD8$^+$ T cell responses without CD4$^+$ T cell help result in chronic persistent HCV infection. CD8$^+$ T cell responses of narrow specificity can easily lose their antiviral activity as a result of virus escape mutations. During chronic infection, prolonged exposure to virus antigens causes the functional exhaustion of CD8$^+$ T cells, which is characterized by the upregulation of various inhibitory receptors such as PD1. Blockade of the PD1–PDL1 pathway restores the function of exhausted T cells, and the combinatorial blockade of inhibitory receptors further reinvigorates the exhausted CD8$^+$ T cells. A recent study reported that direct-acting antiviral (DAA) therapy partially rescues the exhausted T cells[155], although this needs to be confirmed by additional studies. The key features of T cell responses in chronic HCV infection are also observed in chronic HBV infection. CTLA4, cytotoxic T lymphocyte antigen 4; IFN, interferon; PD1, programmed cell death protein 1.

$CD4^+$ T cells were related to $CD8^+$ T cell dysfunction in patients with acute HCV infection who went on to develop chronic infection[101].

Acute HBV infection. More than 90% of adults with acute HBV infection clear the virus, whereas vertical transmission from mothers to their children results in chronic infection in approximately 90% of the exposed children[2]. In acute HBV infection in adults, T cell responses seem to be delayed, which is also the case for acute HCV infection. However, the logarithmic increase in HBV titres occurs much later than the increase in HCV titres during acute infection[11] (FIG. 1), which indicates that HBV-specific T cell responses are not as delayed in terms of the virus kinetics as are the HCV-specific T cell responses.

Similarly to acute HCV infection, virus-specific T cell responses are crucial for the spontaneous resolution of acute HBV infection. HBV-specific T cell responses are vigorous, broad and polyclonal in patients with resolved infection, whereas the T cell responses are relatively weak, narrow and late to be induced in patients with persistent viraemia (reviewed in REF. 102). The crucial role of $CD8^+$ T cells in virus clearance during acute HBV infection was clearly shown by a chimpanzee study that demonstrated that the absence of $CD8^+$ T cells resulted in prolonged infection and a delayed onset of virus clearance[86]. In addition, a series of studies using chimpanzees and HBV-transgenic mice have suggested that virus clearance is mediated by both non-cytolytic and cytolytic effector functions of $CD8^+$ T cells[2].

In contrast to HCV infection, it is controversial whether escape mutations accumulate in $CD8^+$ T cell epitopes of HBV through selection pressure. An early study showed that mutational immune escape is uncommon in HBV infection[103], although a recent study has identified evidence that HLA class I-restricted epitopes in HBV core protein are under selection pressure[104].

HBV-specific $CD4^+$ T cells are also important in determining the outcome of acute HBV infection. HBV-specific $CD4^+$ T cells from patients who cleared acute HBV infection have vigorous effector function (for example, robust IFNγ production) and are specific for multiple epitopes compared with those from patients who developed chronic infection[105]. Moreover, a chimpanzee study showed that the depletion of $CD4^+$ T cells abrogated the function of $CD8^+$ T cells and resulted in chronic HBV infection[106].

Acute HAV infection. A crucial role for HAV-specific $CD8^+$ T cells in acute HAV infection has been postulated for more than two decades[4]. Indeed, $CD8^+$ T cell responses that target multiple epitopes of HAV have been observed and HAV-specific $CD8^+$ T cells detected by HLA-A2 tetramers have an activated phenotype in patients with acute HAV infection[107]. However, a chimpanzee study showed that HAV-specific $CD8^+$ T cells were either undetectable or not functional in the blood during acute HAV infection. Instead, polyfunctional HAV-specific $CD4^+$ T cells were detected when virus titres began to decline[108]. Moreover, HAV-specific $CD4^+$ T cells, but not $CD8^+$ T cells, increased in number in response to a transient virus resurgence detected by faecal HAV shedding[108]. Taken together, these results suggest that acute HAV infection might be controlled by virus-specific $CD4^+$ T cells rather than by $CD8^+$ T cells in chimpanzees, although additional studies are required. In a comparison of chimpanzee studies, the appearance of virus-specific T cells occurred earlier (by 4–6 weeks) during acute HAV infection than during acute HCV infection, despite the fact that titres of both viruses rapidly increased during acute infection[87,88,108].

During acute HAV infection, the frequency of T_{reg} cells in the blood is decreased by FAS (also known as CD95)-mediated apoptosis[109]. In addition, the suppressive function of T_{reg} cells can be directly inhibited by the binding of HAV particles to T_{reg} cells through the immunoregulatory receptor TIM1 (also known as HAVCR1)[110].

Chronic hepatitis virus infection. Although virus-specific T cells might be present in the liver during chronic infection, viruses (particularly HCV) can escape from these T cell responses through mutation. If T cell epitopes are present without mutations, T cells become exhausted and functionally impaired during chronic infection because of sustained antigenic stimulation (FIG. 3). T cell exhaustion is the primary explanation for T cell dysfunction in chronic HCV or HBV infection[1].

T cell exhaustion has been extensively studied in chronic HCV infection[111]. Prolonged exposure to virus antigens is the main cause for T cell exhaustion, which is characterized by the upregulation of inhibitory receptors on T cells, including PD1 (programmed cell death protein 1), CTLA4 (cytotoxic T lymphocyte antigen 4), TIM3, KLRG1 (killer cell lectin-like receptor G1), CD160 and 2B4 (also known as CD244)[112–116], and by high levels of expression of CD39 (REF. 117). Intrahepatic HCV-specific $CD8^+$ T cells in patients with chronic HCV infection are characterized by the co-expression of multiple inhibitory receptors[112,118,119]. The *in vitro* blockade of the PD1–PDL1 pathway rescues the exhausted $CD8^+$ T cells from patients with chronic infection and restores the effector functions of these cells[113,120] (FIG. 3). However, PD1–PDL1 blockade alone is not sufficient to fully restore T cell functions in chronic HCV infection, and the combinatorial blockade of inhibitory receptors such as CTLA4, TIM3 and/or 2B4 with PD1 seems to be required for the efficient reinvigoration of exhausted HCV-specific $CD8^+$ T cells[112,118,119,121] (FIG. 3). Indeed, *in vivo* studies show a limited therapeutic effect of the administration of PD1-specific antibody alone in patients and chimpanzees with chronic HCV infection[122,123].

In chronic HBV infection, inhibitory receptors such as PD1, TIM3, CTLA4 and 2B4 are also highly expressed on exhausted virus-specific $CD8^+$ T cells[124–128]. Moreover, blockade of the PD1–PDL1 pathway partially restores the proliferative capacity and cytokine secretion of these $CD8^+$ T cells[125,129]. Interestingly, CD137 activation in addition to blockade of the PD1–PDL1 pathway further restores the *ex vivo* function of the virus-specific T cells of patients with chronic HBV infection, but not of patients

HBV-transgenic mice
Mice generated by introducing full-length hepatitis B virus (HBV) DNA into the mouse genome. HBV DNA stably replicates in the hepatocytes of these mice at levels comparable to those in the infected livers of patients with chronic HBV infection.

T cell exhaustion
A state of T cell unresponsiveness that is mainly caused by persistent exposure to high levels of antigen, which is characterized by high levels of expression of inhibitory receptors such as PD1. Exhausted T cells have impaired proliferation and cytokine secretion, which abolishes their antiviral activity.

Table 2 | **Summary of immune responses in acute HAV, HBV and HCV infections**

Immune response	HAV	HBV	HCV
Type I and type III IFN responses			
Virus-sensing receptors	MDA5	RIG-I (genotypes A, B and C) and MDA5 (genotype D)	RIG-I, MDA5, PKR and TLR3
Interference mechanisms	• 3ABC destroys MAVS • 3CD cleaves TRIF • 3C cleaves NEMO	• Polymerase blocks IRF3 activation • HBx downregulates MAVS	• NS3–NS4A cleaves MAVS • NS3–NS4A cleaves TRIF
ISG induction in the infected liver*	Minimal induction of ISG expression	No induction of ISG expression	Increased expression of many ISGs
Antibodies			
Neutralizing antibodies	Protective immunity by HAV-specific antibodies	Protective immunity by HBsAg-specific antibodies	HCV easily escapes from the neutralizing activity of E1- and E2-specific antibodies
Vaccines inducing neutralizing antibodies	Inactivated virus	Recombinant HBsAg	No approved vaccine
T cells			
Timing*	From 4 to ~6 weeks after infection	Variable depending on infection dose	8–12 weeks after infection
Relation to the outcome of infection	Not determined	Vigorous and broad (multiple epitope-specific) T cell responses lead to spontaneous resolution of infection	Vigorous and broad (multiple epitope-specific) T cell responses lead to spontaneous resolution of infection
$CD4^+CD25^+FOXP3^+$ T_{reg} cells	Decreased by FAS-mediated apoptosis	Not determined	Increase transiently

HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, interferon; IRF, IFN response factor; ISG, IFN-stimulated gene; MAVS, mitochondrial antiviral signalling protein; MDA5, melanoma differentiation-associated protein 5; NEMO, NF-κB essential modulator; PKR, protein kinase R; RIG-I, retinoic acid-inducible gene I; TLR3, Toll-like receptor 3; T_{reg} cell, regulatory T cell; TRIF, Toll/IL-1 receptor domain-containing adaptor inducing IFNβ. *Based on data from chimpanzee studies[11,88,108].

with chronic HCV infection[130], which indicates that the mechanisms of T cell exhaustion might differ between HBV infection and HCV infection. Furthermore, IL-12 treatment combined with PD1 blockade further restores the function of exhausted HBV-specific $CD8^+$ T cells *in vitro*[131]. A recent study showed that T-bet (also known as TBX21) expression is lacking in dysfunctional $CD8^+$ T cells in chronic HCV infection and HBV infection and that exogenous IL-12 in addition to T-bet induction restores the cytokine production of these $CD8^+$ T cells[132].

In addition to being exhausted, virus-specific $CD8^+$ T cells are deleted during chronic HBV infection. HBV-specific T cells of patients with chronic infection are susceptible to apoptosis as a result of the increased expression of BIM or TRAILR2, which is targeted by $TRAIL^+$ liver NK cells[133,134]. This is an important example of how NK cells can negatively regulate virus-specific $CD8^+$ T cells during chronic HBV infection[134].

T cell functions are also suppressed by $CD4^+CD25^+FOXP3^+$ T_{reg} cells. During chronic HCV infection and HBV infection, the number of T_{reg} cells tends to increase in the blood and liver[135–138], and the frequency of these cells in the blood is positively correlated with virus load[135,138], which indicates that T_{reg} cells contribute to virus persistence by suppressing virus-specific effector T cells. In chronic HCV infection, the suppression of effector T cells by T_{reg} cells occurs through a direct cell-to-cell contact mechanism independently of the secretion of IL-10 and TGFβ[139]. Interestingly, intrahepatic T_{reg} cells express increased levels of PD1 in patients with chronic HCV infection, and the proliferation and suppressive functions of these T_{reg} cells are inhibited by PDL1 through the attenuation of STAT5 phosphorylation[140].

Comparing the immune responses

As described above, acute HCV infection tends to progress to chronic persistent infection, whereas acute HBV infection spontaneously resolves in more than 90% of adult patients. Acute HAV infection also spontaneously resolves and does not progress to chronic infection. In acute HCV infection and HBV infection, spontaneous resolution is achieved through robust and broad $CD8^+$ T cell responses, which are helped and maintained by $CD4^+$ T cells. In addition to T cells, HBsAg-specific neutralizing antibodies, the production of which depends on $CD4^+$ T cell help, have a crucial role in the resolution of HBV infection and in lifelong protective immunity. However, the role of HCV-specific antibodies in the control of HCV infection is controversial. In acute HAV infection, neutralizing antibodies control the virus and confer lifelong protective immunity to hosts after resolution. Moreover, polyfunctional $CD4^+$ T cell responses seem to be important for the control of HAV, whereas the role of $CD8^+$ T cells is controversial. The immune response that is elicited by each type of hepatitis virus is summarized in TABLE 2, and the differences in immune responses to these

Box 3 | **Recent progress in the development of a prophylactic HCV vaccine**

Prophylactic hepatitis C virus (HCV) vaccine candidates have been developed to elicit HCV-specific memory T cells and/or broadly neutralizing antibodies. In principle, only neutralizing antibodies can protect hosts from infection itself[168]. However, virus-specific memory T cells can prevent the evolution of the infection to chronic persistent infection.

Evidence that emphasizes the crucial role of T cell responses in protective immunity to HCV infection (including reinfection) has accumulated from studies in patients and chimpanzees (reviewed in REF. 90). In particular, HCV-specific memory T cells protect HCV-rechallenged chimpanzees and naturally reinfected patients, as shown by lower levels of the virus and a shorter duration of viraemia[79,169], which supports the rationale of developing T cell-based vaccines. Viral vectors (including adenovirus, modified vaccinia virus Ankara (MVA), vaccinia virus and others), virus-like particles, recombinant DNA plasmids and yeast vectors that express HCV proteins have been shown to induce HCV-specific T cell responses that mediate protective immunity in chimpanzees[168,170,171]. The key immunological factors for successful vaccine-induced protective immunity are the early proliferation of polyfunctional $CD8^+$ T cells upon HCV infection and the high levels of expression of the memory precursor marker CD127 (REF. 172). In a recent Phase I trial in healthy human volunteers, a simian adenoviral vector expressing HCV nonstructural proteins primed HCV-specific $CD4^+$ and $CD8^+$ T cells, and a boost immunization with MVA expressing the same HCV proteins elicited sustained memory and effector T cell responses with enhanced functionality and proliferative capacity[173]. This vaccine is currently proceeding to a Phase II trial.

There is also evidence to support a role of broadly neutralizing antibodies in the control of HCV infection[78,79]. In addition, cross-reactive neutralizing antibodies are associated with protection against chronic infection in the setting of reinfection of injecting drug users[174]. Moreover, broadly neutralizing antibodies protected against a heterologous HCV challenge or abolished established HCV infection in a chimeric mouse model[82,83]. As an antibody-based prophylactic vaccine, a recombinant E1–E2 vaccine induced broadly neutralizing antibodies in chimpanzees challenged with homologous or heterologous HCV strains[175]. The generation of cross-genotype neutralizing antibodies was also reported in healthy volunteers in clinical trials using recombinant E1–E2 proteins plus adjuvant[77].

viruses may explain the tendency to resolution of acute infection versus progression to chronic infection.

The intrahepatic IFN response is strong and sustained in acute HCV infection, whereas it is weak in acute HAV infection and HBV infection (FIG. 1; TABLE 2). However, the IFN response is not sufficient for the spontaneous resolution of acute HCV infection. Rather, the strong and sustained production of type I IFNs may hamper the induction of virus-specific T cell responses, as shown in mouse models of lymphocytic choriomeningitis virus infection[141,142]. Indeed, the induction of virus-specific T cell responses is markedly delayed in acute HCV infection despite the early increase in the HCV load[88]. In acute HBV infection, virus-specific T cell responses are not as delayed in relation to the delayed increase in virus titre, and the appearance of virus-specific T cells in acute HAV infection occurs earlier than that in acute HCV infection by 4–6 weeks (FIG. 1). In addition to the weaker IFN response, other mechanisms to explain why T cell responses are not delayed in HAV infection include the fact that HAV is released from infected cells in a non-enveloped form[143]. Delayed T cell responses during acute HCV infection may allow the virus more time to mutate and thus easily escape from the neutralizing activity of HCV E1- and E2-specific antibodies and from T cell recognition. In addition, the number of T_{reg} cells tends to increase during acute HCV infection[98–100], whereas these cells decrease during acute HAV infection[109], which indicates that the larger T_{reg} cell population might contribute to the suppression of HCV-specific T cells in acute HCV infection. In summary, the characteristic immune responses that are elicited by HCV may explain why HCV infection often progresses to chronic persistent infection.

Immune-mediated liver injury

Hepatitis virus infection results in liver injury, which is clinically manifested by increased serum levels of liver enzymes such as alanine aminotransferase (ALT; also known as SGPT), and fulminant liver injury develops in extreme cases. Acute HCV infection is often subclinical, whereas acute HAV or HBV infection tends to result in symptomatic hepatitis in adults. Liver injury is not directly caused by the hepatitis virus but is instead caused by immune-mediated mechanisms[2], and hence the type, timing and extent of the immune response that is induced by the different viruses have implications for pathology.

Virus-specific T cells. The important role of T cells in mediating liver injury is supported by findings that the appearance of T cells in the liver tends to coincide with an increase in serum ALT levels during acute HAV, HBV and HCV infections[1,2,4]. For example, in chimpanzees with acute HCV infection, the induction of HCV-specific $CD8^+$ T cells, which is delayed, and their recruitment to the liver occur simultaneously with peaks of serum ALT levels, as well as a decrease in virus titres[88].

Virus-specific $CD8^+$ T cells can contribute to both virus control and liver injury in hepatitis virus infection. Among the effector functions of $CD8^+$ T cells, IFNγ is thought to be a non-cytolytic antiviral cytokine[68,69]; thus, it can eliminate viruses without severe injury to the liver, in contrast to the cytotoxic activity of $CD8^+$ T cells[2]. In addition to $CD8^+$ T cells, $CD4^+$ T cells have a role in liver injury. In particular, both HBV and HCV infections promote T_H17 cell differentiation. T_H17 cells contribute to liver injury by producing IL-22 during chronic HBV infection or HCV infection[144–148].

Antigen-nonspecific cells. $CD8^+$ T cell-mediated mechanisms of liver injury were first examined in a series of studies that used HBV-transgenic mice. The adoptive transfer of HBV-specific $CD8^+$ T cells into liver-specific HBV-transgenic mice causes acute necroinflammatory liver injury, which histologically resembles acute viral hepatitis in humans (reviewed in REFS 2,149). In this model, HBV-specific $CD8^+$ T cells directly induce the apoptotic death only of hepatocytes in close proximity. Liver injury is greatly amplified by antigen-nonspecific mononuclear cells, which are recruited to the liver by the HBV-specific $CD8^+$ T cells through the production of CXCL9 and CXCL10 (REF. 149).

It has not been clearly shown whether antigen-nonspecific mononuclear cells have a role in liver injury during hepatitis virus infection in humans. However, as described above, virus-specific $CD8^+$ T cells are exhausted in chronic HCV infection or HBV infection, which indicates that antigen-nonspecific cells rather than virus-specific cells must contribute to liver injury during chronic infection. In fact, non-HBV-specific T cells were predominantly observed in inflamed, HBV-infected livers

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous population of myeloid cells that expands in pathological conditions, such as chronic virus infections and cancer, as a result of altered haematopoiesis. MDSCs suppress the function of various immune cells, including $CD4^+$ and $CD8^+$ T cells and natural killer cells.

compared with non-inflamed, HBV-infected livers[150], whereas the size of the HBV-specific $CD8^+$ T cell infiltrate was comparable between the two types of liver[150].

Immunoregulatory cells. Immune-mediated host injury can be regulated by $FOXP3^+$ T_{reg} cells. A role for $FOXP3^+$ T_{reg} cells in the regulation of liver injury has been demonstrated in acute HAV infection. As described above, the number of T_{reg} cells is decreased by FAS-mediated apoptosis during acute HAV infection; importantly, the number of T_{reg} cells and the suppressive activity of the T_{reg} cell population are inversely correlated with the degree of liver injury, which indicates that reduced suppressive activity of the T_{reg} cell population results in severe liver injury during acute HAV infection[109].

Another type of suppressor cell, known as myeloid-derived suppressor cells (MDSCs), was recently studied in HBV infection. The number of arginase-expressing granulocytic MDSCs was increased in HBV infection and these cells regulated liver immunopathology[151]. MDSCs are also induced in HCV infection, and they suppress T cells through the production of reactive oxygen species[152], and suppress NK cells through the expression of arginase[153].

Clinical prospects

Recently, DAAs have been introduced for the treatment of HCV infection, and the proportion of patients with a sustained virological response has markedly increased. However, the accessibility of DAA treatment is limited because of its high cost as well as the fact that many patients are unaware of their HCV infection. In addition, there is a chance of re-infection after successful DAA treatment of HCV infection[154], although a recent study reported that DAA therapy partially rescues the exhausted T cells[155]. Therefore, HCV control at the population level must depend on a prophylactic vaccine. The development of such a vaccine will require understanding why T cell responses are delayed during acute HCV infection and the role of neutralizing antibodies in the control of HCV infection. Recent progress in the development of a prophylactic HCV vaccine is described in BOX 3.

For the treatment of chronic HBV infection, nucleoside and nucleotide analogue antivirals have been successfully used to suppress virus replication. The decreased serum virus load in response to nucleoside and nucleotide analogues progressively restores HBV-specific T cell responses[156,157]. However, HBV exists in cells as cccDNA, which cannot be eliminated using nucleoside and nucleotide analogues. Therefore, a practical goal of HBV therapeutics has been HBs seroconversion (the loss of HBsAg and the development of HBsAg-specific antibodies), which occurs during spontaneous recovery from acute HBV infection. As described above, HBsAg-specific antibodies can control HBV infection even in the presence of cccDNA. Currently, peg-IFNα is used to induce HBs seroconversion in patients with chronic HBV infection; however, the efficacy is not satisfactory[158]. In the future, other immunological therapeutics must be considered for achieving HBs seroconversion, including therapeutic vaccines and immune checkpoint blockade. Immunological therapeutics may also decrease the cccDNA load, as IFNγ and TNF are known to reduce the level of cccDNA[68].

Furthermore, comparison studies that investigate the similarities and differences in immune responses that are elicited by HAV, HBV and HCV will help us to understand the mechanisms of virus persistence. In particular, understanding the immune responses in acute HAV infection may aid the development of a prophylactic HCV vaccine, and understanding immune dysfunction in chronic HBV and HCV infections will facilitate the development of novel HBV therapeutics.

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Competing interests statement
The authors declare no competing interests.