IMMUNOLOGY OF HEPATITIS B VIRUS AND HEPATITIS C VIRUS INFECTION

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Abstract | More than 500 million people worldwide are persistently infected with the hepatitis B virus (HBV) and/or hepatitis C virus (HCV) and are at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. Despite many common features in the pathogenesis of HBV- and HCV-related liver disease, these viruses markedly differ in their virological properties and in their immune escape and survival strategies. This review assesses recent advances in our understanding of viral hepatitis, contrasts mechanisms of virus–host interaction in acute hepatitis B and hepatitis C, and outlines areas for future studies.

NECROINFLAMMATORY
A state in which there is morphological evidence of infiltration of inflammatory cells and necrosis of parenchymal cells.

PROTECTIVE IMMUNITY
The immune responses of individuals who have recovered from a primary infection and, on re-exposure to the pathogen, are protected from developing severe disease and chronic infection. Protective immunity can be sterilizing if it protects from a productive infection.

PSEUDOTYPE PARTICLE
A viral particle containing the genome of one virus in the envelope of another virus.

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the most common causes of liver disease worldwide. Both viruses can be transmitted parenterally, sexually and perinatally, with perinatal and sexual transmission being more common for HBV than for HCV (TABLE 1 and online supplementary information S1 (table)).

Although both viruses induce immune-mediated acute and chronic NECROINFLAMMATORY liver disease, the natural history and outcome of HBV and HCV infection differ profoundly. Whereas vertical transmission of HBV from mother to neonate always results in chronic hepatitis, infection during adulthood typically does not; instead, it results in lifelong PROTECTIVE IMMUNITY[1]. By contrast, HCV readily establishes chronic hepatitis in 60–80% of infected adults[2], with slightly higher clearance rates reported only for genotype 2 HCV in Africa[3]. Because a vaccine against infection with HCV does not exist and because there is no cure for most patients who already have chronic hepatitis B, it is crucial to study components of successful immune responses, viral strategies for immune evasion and mechanisms of disease pathogenesis.

Our understanding of the early phase of HBV and HCV infection has been considerably advanced by recent prospective studies in chimpanzees, the only animal that can be infected with HBV and HCV[4–15] (BOX 1). In addition, much has been learned from infections with HBV-related hepadnaviruses (such as woodchuck hepatitis virus and duck hepatitis virus) in their respective native hosts (reviewed in REF. 16) and from transgenic mice that have replication-competent copies of HBV genomes in their hepatocytes[17]. An infectious molecular clone of the HCV-related GB virus B hepatitis agent, derived from patient GB and infectious to tamarins, has recently been developed to create an *in vivo* surrogate model for hepatitis C[18]. For *in vitro* studies of HCV biology, several experimental systems have now become available that allow analysis of HCV replication and polyprotein processing [19,20], infection of hepatoma cells with HCV PSEUDOTYPE PARTICLES[21,22] and neutralization of pseudotype-particle infection by antibodies. Models for studying the production of hepatitis C virions *in vitro* are being developed at present[23]. It is to be hoped that these developments open new avenues for the generation of vaccines to prevent HCV infection, which are not available at present, and effective immunotherapies to resolve chronic hepatitis resulting from HBV or HCV infection.

This article reviews our current knowledge of virus–host interactions in hepatitis B and hepatitis C. The review starts with a brief description of the virological and clinical features of HBV and HCV infection, which is followed by a detailed characterization of those immune responses that are associated with clinical recovery and protective immunity. The final sections describe the viral immune-evasion mechanisms that are implicated in the development of persistent infection and the immunological characteristics of chronic hepatitis.

HEPADNAVIRIDAE
A family of hepatotropic DNA viruses, which contain double-stranded DNA genomes and causes hepatitis in humans and animals. Hepadnaviruses have very small genomes of relaxed circular, partially double-stranded DNA. They replicate through an RNA intermediate, which they translate back into DNA using reverse transcriptase. Hepadnaviruses include hepatitis B virus, duck hepatitis virus, heron hepatitis B virus, ground squirrel hepatitis virus and woodchuck hepatitis virus.

Molecular virology of HBV and HCV

When discussing virus–host interactions, it is important to note that HBV, a member of the HEPADNAVIRIDAE family[16], and HCV, which constitutes a separate genus in the FLAVIVIRIDAE family[24], differ considerably in their genomic organization and replication strategies (TABLE 2 and online supplementary information S2 (table)).

HBV. The HBV genome is a relaxed circular DNA of ~3,200 nucleotides and consists of a full-length of negative strand and a shorter positive strand (FIG. 1a). The 5′ end of the negative strand is covalently linked to the viral reverse transcriptase, whereas the 5′ end of the positive strand bears an oligoribonucleotide. After virions enter hepatocytes, by an as-yet-unknown receptor, NUCLEOCAPSIDS transport their cargo, the genomic HBV DNA, to the nucleus, where the relaxed circular DNA is converted to COVALENTLY CLOSED CIRCULAR DNA (cccDNA) (FIG. 2a). The cccDNA functions as the template for the transcription of four viral RNAs (FIG. 1a), which are exported to the cytoplasm and used as mRNAs for translation of the HBV proteins. The longest (pre-genomic) RNA also functions as the template for HBV replication, which occurs in nucleocapsids in the cytoplasm (reviewed in REF. 16) (FIG. 2a). Some of the HBV DNA and polymerase-containing capsids are then transported back to the nucleus, where they release the newly generated relaxed circular DNA to form additional cccDNA. Others are enveloped by budding into the endoplasmic reticulum and secreted after passing through the Golgi complex. In addition to 42–47-nm virions, the blood of HBV-infected patients contains 20-nm spheres that consist of HBV surface antigen (HBsAg) and host-derived lipids. These spheres outnumber the virions by a factor of 10^4–10^6.

Table 1 | **Clinical features of hepatitis B and hepatitis C**

Feature	Hepatitis B	Hepatitis C
Public-health impact		
Worldwide	350 million people infected	170 million people infected
United States	1 million people infected; ~5,000 deaths per year	4 million people infected; leading cause of liver transplantation
Clinical course of infection		
Vertical (or perinatal) transmission	Most common from mother to neonate, followed by childhood infection	Rare
Horizontal transmission	Intravenous drug use, parenteral, sexual	Intravenous drug use, parenteral, sexual
Vertical (or perinatal) transmission: infection outcome	90% of individuals have chronically evolving hepatitis	–
Horizontal transmission: infection outcome	90% of individuals recover	60–80% of individuals have chronically evolving hepatitis; except those infected with genotype 2 HCV in Africa, which is cleared by 53% of individuals
Characteristic histological features of chronic hepatitis	Ground-glass inclusions of HBsAg in hepatocytes, appearing as pale, eosinophilic areas in the cytoplasm but not the nucleus	Lymphoid aggregates with organization similar to primary lymphoid follicles; steatosis (with genotype 3 HCV); reactive epithelial changes of bile ducts
Disease progression		
Liver cirrhosis	2–5 per 100 person years in HBeAg-positive patients (genotype C HBV associated with higher risk than genotype B)	5–10% after 10 years of infection
Hepatocellular carcinoma (HCC)	5-year cumulative HCC incidence in patients with cirrhosis in Western countries is 5%; 5-year cumulative HCC incidence in patients with cirrhosis in Asia is 16%; 0.2 per 100 person years in asymptomatic HBsAg carriers; 0.1 per 100 person years in untreated patients without cirrhosis; 3–8 per 100 person years in Asian patients with compensated cirrhosis	5-year cumulative HCC incidence in patients with cirrhosis in Western countries is 17%; 5-year cumulative HCC incidence in patients with cirrhosis in Japan is 30%; 3.7 per 100 person years in patients with cirrhosis in Europe and the United States; 7.1 per 100 person years in patients with cirrhosis in Japan
Preventive vaccination	Yes (using recombinant HBsAg), induces neutralizing HBsAg-specific antibodies and $CD4^+$ and $CD8^+$ T cells; vaccination of neonates prevents persistent infection	No (not available)
Therapy for persistent infection	Interferon-α, lamivudine or adefovir dipivoxil; frequent development of lamivudine escape mutations; rarely leads to HBV clearance	Pegylated interferon-α and ribavirin combination; HCV clearance in 45–80% of individuals, depending on HCV genotype

References are provided in the online version of this Table (see online supplementary information S1). HBV, hepatitis B virus; HCV, hepatitis C virus; HBeAg, HBV e antigen; HBsAg, HBV surface antigen.

HCV. The HCV genome is a single-stranded RNA of positive polarity of ~10,000 nucleotides. The RNA encodes a long open reading frame flanked by two untranslated regions (UTRs) (FIG. 1b) that contain signals for viral protein and RNA synthesis and for the coordination of both processes (reviewed in REF. 24). In contrast to HBV, the HCV genome does not enter the nucleus of infected cells. Instead, HCV RNA functions directly as an mRNA in the cytoplasm of the host cell, where translation is initiated through an INTERNAL RIBOSOMAL ENTRY SITE in the 5′ UTR. The translated polyprotein is co- and post-translationally processed by cellular and viral proteases into structural proteins (core, envelope protein 1 (E1) and E2), p7 and non-structural proteins (NS2, -3, -4A, -4B, -5A and -5B) (reviewed in REF. 24) (FIG. 1b). Following synthesis and maturation, non-structural proteins and viral RNA form membrane-associated replication complexes, which appear as a perinuclear membranous web[25] (FIG. 2b). These replication complexes then catalyse the transcription of negative-strand RNA intermediates from which, in turn, progeny positive-strand RNA molecules are generated[24]. Capsid proteins and genomic RNA assemble into a nucleocapsid and bud through intracellular membranes into cytoplasmic vesicles. With the recent development of an *in vitro* model of HCV-virion production and release[23], the analysis of this final part of the viral life cycle is an exciting area for future research.

Box 1 | **Use of the chimpanzee model for the study of viral hepatitis**

Examples of the successful use of the chimpanzee model
- The chimpanzee model contributed to our understanding of viral hepatitis B and hepatitis C as transmissible diseases.
- The chimpanzee model has been used to generate hepatitis B virus (HBV) and hepatitis C virus (HCV) challenge pools and to determine their infectivity titres.
- The chimpanzee model has been used to assess the infectivity of molecular HCV clones and to confirm the relevance of specific genetic elements in the viral life cycle.
- The chimpanzee model has been used to assess the neutralization capacity of HBV- and HCV-specific antibodies.
- Protective immunity has been assessed by rechallenge (with homologous or heterologous virus) of chimpanzees that have recovered from infection.
- Viral nucleotide- and amino-acid-substitution rates have been determined in HBV- and HCV-infected chimpanzees.
- Antibody and T-cell escape mutants have been identified in HBV-infected and HCV-infected chimpanzees.

Advantages of the chimpanzee model
- The chimpanzee is the only animal model for immunological studies of the natural course of HBV and HCV infection.
- Chimpanzees can be infected with defined inocula and studied in the early phase of infection.
- Studies in chimpanzees are carried out in an unselected population, because all exposed animals can be analysed. By contrast, human studies are biased towards individuals who present with clinical symptoms, and these studies typically do not include those individuals who remain asymptomatic, do not develop antibodies or lose antibodies after clinical recovery. This is one possible explanation for the observation that the clinical recovery rate is higher in chimpanzee studies than in human studies[2,8].
- The prospective analysis of intrahepatic immune responses is possible in chimpanzees, because sequential liver biopsies can be carried out throughout the course of infection.

Disadvantages of the chimpanzee model
- Ethical considerations limit biomedical research on chimpanzees and other primates.
- Owing to high costs and limited availability of chimpanzees for research, many studies are limited to two to three animals.
- Vertical transmission, the main route of HBV transmission in humans, is rare in chimpanzees.
- The clinical course of hepatitis is milder in chimpanzees than in humans.
- The humoral immune response is weaker and more restricted in chimpanzees than in humans.

Considerations for immunological studies
- Chimpanzee DNA has 98–99% sequence similarity to human DNA, and it is possible to use the same reagents and tests as in human studies. Moreover, many antibodies have been specifically evaluated for immunological studies of chimpanzees.
- There are differences in both MHC class I and II sequence and diversity between chimpanzees and humans. However, several HLA lineages are preserved, and chimpanzee orthologues of human HLA alleles have been identified. Many HBV- and HCV-derived peptides are presented by both human and chimpanzee MHC molecules and recognized by both human and chimpanzee T cells.

Acute HBV and HCV infection of adults

HBV. In a typical case of acute infection with HBV, HBV DNA is detectable in the circulation (using PCR) within 1 month of infection, but it remains at the relatively low level of 10^2–10^4 genome equivalents per ml for up to 6 weeks before the HBV DNA and the secreted HBV e ANTIGEN (HBeAg) and HBsAg increase to their peak titres (FIG. 3a). HBV core antigen (HBcAg)-specific IgM appears early, and HBcAg-specific IgG persists for life, irrespective of the outcome of infection (FIG. 3a). Approximately 10–15 weeks after infection, serum ALANINE AMINOTRANSFERASE (ALT) levels start to rise, which is indicative of T-cell-mediated liver injury. Interestingly, most of the HBV DNA in the serum and the liver can be cleared before the ALT peak, as shown in experimentally infected chimpanzees[4]. More than 90% of acutely infected adults resolve all clinical symptoms, develop HBeAg- and HBsAg-specific antibodies, clear free HBeAg and HBsAg from the circulation and maintain lifelong protective immunity. Despite complete clinical recovery, however, trace amounts of HBV DNA persist and are controlled by humoral and cellular immune responses. In contrast to HBV infection during adulthood, perinatal HBV infection typically results in chronic hepatitis. Its clinical course is not the focus of this review and is therefore only briefly outlined in FIG. 3b.

HCV. In contrast to HBV, HCV reaches high serum titres within 1 week of infection[26,27]. Adaptive cellular immune responses are delayed by at least 1 month, and humoral immune responses by at least 2 months, in both humans and chimpanzees, raising the hypothesis that the virus 'outpaces' the adaptive immune response[15,26]. Accordingly, clinical symptoms such as jaundice, which are attributed to T-cell-mediated liver injury and are common in acute hepatitis B, are rarely observed in infection with HCV. After the first weeks

Table 2 | **Virology of HBV and HCV**

Viral features	HBV	HCV
Molecular virology		
Structure	42 nm; enveloped nucleocapsid; partially double-stranded DNA genome	50 nm; enveloped nucleocapsid; positive-stranded RNA genome
Family	Hepadnaviridae family	Flaviviridae family; hepacivirus genus
Receptor	Unknown; there are several candidate HBV-binding proteins	Unknown; the receptor complex probably includes the tetraspanin CD81 and as-yet-unknown hepatocyte-specific factors; there are several other candidate HCV-binding proteins
Replication strategy	Replication of HBV DNA occurs by reverse transcription of an RNA intermediate within cytoplasmic nucleocapsids	Replication occurs by synthesis of a genome-length minus-strand RNA intermediate within cytoplasmic replication complexes that form a perinuclear membranous web
Mutation rate	Low (1 in 100,000 bases per year)	High (1 in 1,000 bases per year)
Genotypes	8 genotypes (8% intergroup divergence)	6 main genotypes (20–35% overall sequence difference); more than 50 subtypes (10–25% difference); quasispecies in every infected patient
Integration into host chromosome	Yes	No
Viral kinetics		
Viral half-life	2–3 days	3 hours
Viral production	10^{10}–10^{12} virions per day	10^{12} virions per day

References are provided in the online version of this Table (see online supplementary information S1). HBV, hepatitis B virus; HCV, hepatitis C virus.

FLAVIVIRIDAE
A family of related positive-strand RNA viruses, which consists of three genera: flaviviruses, pestiviruses and hepaciviruses. Flaviviridae replicate by synthesis of a minus-strand RNA intermediate. Dengue virus, bovine viral diarrhoea virus and hepatitis C virus are examples from the three genera.

NUCLEOCAPSID
A nucleic acid and its surrounding protein coat (or capsid). The nucleocapsid forms the basic structural unit of the virion. Depending on the virus, the nucleocapsid might be a naked core or be surrounded by a membranous envelope.

COVALENTLY CLOSED CIRCULAR DNA
(cccDNA). The double-stranded cccDNA of HBV is the transcriptional template of HBV in the nucleus of infected cells.

INTERNAL RIBOSOMAL ENTRY SITE
(IRES). A well-defined and highly conserved secondary structure located in the 5′ untranslated region of some viral and cellular mRNAs. It mediates the translation initiation of the viral message by a 5′-cap-independent mechanism.

HBV e ANTIGEN
(Hepatitis B virus e antigen, HBeAg). HBeAg is derived from the pre-core polypeptide, which together with the core polypeptide, is encoded by the nucleocapsid open reading frame. After removal of the amino-terminal 29 amino acids of the pre-core polypeptide in the endoplasmic reticulum and trimming of the carboxyl terminus, the remaining polypeptide is secreted from infected cells as HBeAg. Neither pre-core polypeptide nor secreted HBeAg are required for HBV replication.

of infection, the rate of increase in the viral titre slows[27] (FIG. 3c,d), and the typical peak HCV titre remains several logs lower than the peak HBV titre in acute infection. Approximately 8–12 weeks after infection, when serum ALT levels peak, HCV RNA titres decline. HCV-specific antibodies might become detectable around this time, later or not at all, and they do not indicate the outcome of infection. Most patients develop chronic hepatitis with relatively stable viral titres, about 2–3 logs lower than in the acute phase (FIG. 3d). Only a small proportion of patients recover and test negative for HCV RNA using standard diagnostic assays (FIG. 3c). Viral clearance from the liver, and possibly from other reservoirs, probably takes longer than viral clearance from the blood[10], because recurrent viraemia has been observed in a patient[28] and a chimpanzee[9] even after 4–5 months of consistently undetectable viraemia. Whether HCV is ultimately completely eradicated is still a matter of debate and requires further study[29]. Because HCV-specific antibody titres decline and might disappear completely 10–20 years after recovery, complete HCV clearance might be achieved by at least a subgroup of patients[30,31].

Innate immune responses

Microarray analyses of serial liver biopsies of experimentally infected chimpanzees reveal striking differences in the early immune responses to HBV and HCV[10,32,33]. HBV does not induce any detectable changes in the expression of intrahepatic genes in the first weeks of infection[32]. By contrast, HCV induces early changes in the expression of many intrahepatic genes, including genes involved in the type I interferon (IFN) response[10,15,33]. So, HBV seems to avoid the induction of strong innate immune responses during the first weeks of infection[32], but this does not affect the high recovery rate. By contrast, HCV induces vigorous intrahepatic type I IFN responses, but it seems to be resistant to their effects and frequently succeeds in establishing chronic hepatitis.

HBV. Despite these striking differences in the intrahepatic gene-expression patterns in the early phase of HBV and HCV infection, a role for the innate immune response in the control of early HBV replication should not be dismissed, and expression of immune-response genes might occur below the level of detection of the microarray analysis that has been carried out. Notably, most of the HBV DNA can be cleared from the serum and the livers of experimentally infected chimpanzees before a detectable adaptive immune response in the liver[4]. Indeed, antiviral effects of IFN-α and IFN-β (type I IFNs) have been shown in transgenic mice that have chromosomal, replication-competent copies of HBV genomes in their hepatocytes[34]. In this model, IFN-α- and IFN-β-induced mechanisms inhibit the formation of new HBV capsids, destabilize existing capsids and degrade pre-formed HBV RNA[34,35]. This antiviral effect is not mediated by typical IFN-induced proteins — such as myxovirus resistance 1 (MX1), RNase L, IFN-inducible double-stranded-RNA-dependent protein kinase (PKR) or IFN-regulatory factor 1 (IRF1)[36] — and it seems to be proteasome dependent[37]. In addition, in this model, downregulation of HBV replication can be mediated by IFN-γ that is produced by activated natural killer T (NKT) cells[38,39] and T cells[40].

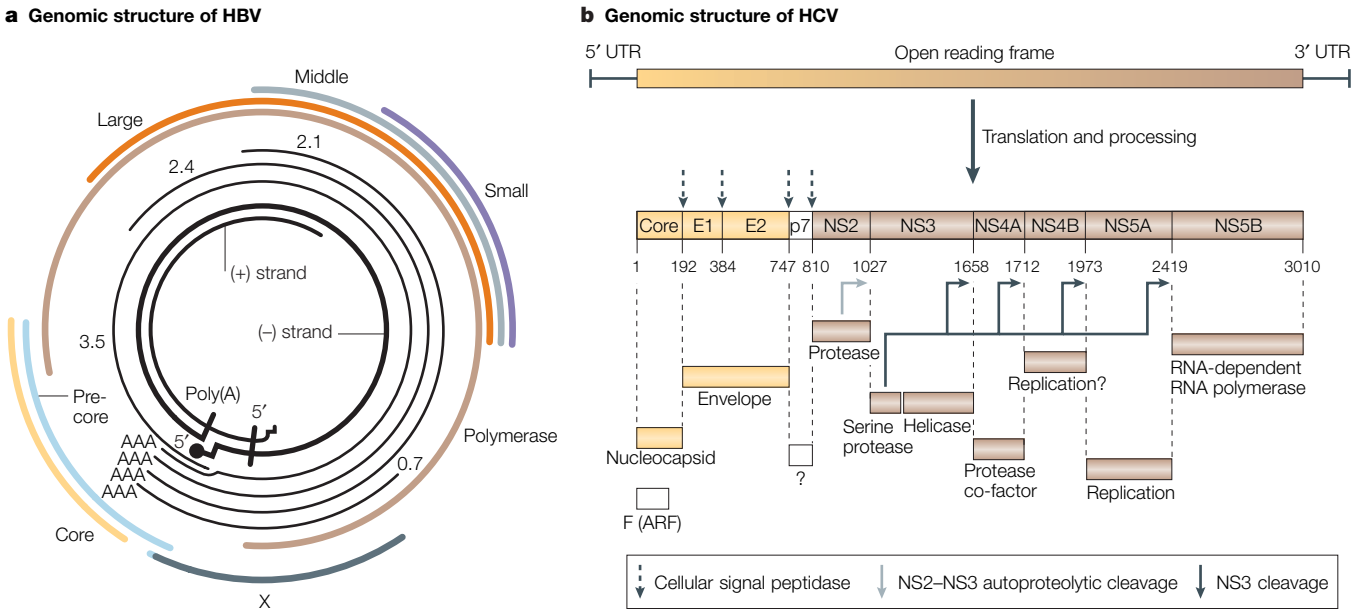

Figure 1 | **Genomic structure and translated proteins of HBV and HCV. a** | The genomic structure of hepatitis B virus (HBV) is shown. The inner circles represent the full-length minus (–) strand (with the terminal protein attached to its 5′ end) and the incomplete plus (+) strand of the HBV genome. The thin black lines represent the 3.5, 2.4, 2.1 and 0.7 kilobase mRNA transcripts, which are all terminated near the poly(A) (polyadenylation) signal. The outermost coloured lines indicate the translated HBV proteins: that is, large, middle and small HBV surface proteins, polymerase protein, X protein, and core and pre-core proteins[16]. **b** | The genomic structure of hepatitis C virus (HCV) is shown. A long open reading frame encodes a polyprotein of ~3,010 amino acids. The numbers below the polyprotein indicate the amino-acid positions of the cleavage sites for cellular and viral proteases. An F (frameshift) protein is translated from a short alternative reading frame (ARF)[24]. E, envelope protein; NS, non-structural protein; UTR, untranslated region.

HCV. In contrast to HBV infection, transcriptional changes in type I IFN-response genes have been shown in the livers of experimentally infected chimpanzees within 1 week of infection[10,15,33]. Although it is known that HCV replication yields double-stranded RNA and although several pathways of induction of IFN-α and IFN-β by double-stranded RNA have recently been identified in other systems[41–43], it is not yet clear whether these pathways also operate in HCV infection. Strikingly, these type I IFN responses in the liver do not correlate with the outcome of infection[10,15,33], even though HCV replicons are highly sensitive to type I IFNs *in vitro*[44]. These findings indicate that HCV might not be sensitive to the antiviral effects of IFN-α and IFN-β *in vivo*. Three candidate mechanisms have been proposed based on *in vitro* model systems. First, the HCV serine protease NS3–NS4A blocks IRF3-mediated induction of type I IFN *in vitro*[45]. Second, specific sequences within E2 and NS5A inhibit PKR *in vitro*. E2 can function as a decoy target for PKR because of its sequence homology to the phosphorylation sites of both the enzyme and its substrate, the EUKARYOTIC TRANSLATION INITIATION FACTOR 2α (EIF2α)[46]. NS5A forms heterodimers with PKR and thereby inhibits its function[47]. Despite this use of cell-culture systems in which HCV proteins are overexpressed, these findings are intriguing because E2 sequences of HCV genotype 1, which is relatively resistant to IFN therapy, inhibit PKR more efficiently than E2 sequences of HCV genotypes 2 and 3, which respond much better to IFN therapy[46]. Similar correlations with the outcome of IFN treatment of HCV-infected patients were also reported for NS5A sequences, but these were limited to specific viral isolates from Japan (reviewed in REF. 48).

Last, specific HCV proteins might interfere with the function of innate effector cells, such as natural killer (NK) cells. A role for NK cells in early HCV infection was recently indicated by a large immunogenetic study in which the presence of a specific NK-cell receptor–HLA COMPOUND GENOTYPE correlated with HCV clearance and clinical recovery[49]. Individuals who were homozygous for *KIR2DL3* (killer-cell immunoglobulin-like receptor 2DL3) and group 1 HLA-C alleles were more likely to recover from HCV infection than individuals with any other KIR–HLA compound genotype. Although a functional correlate has not been identified for this observation, it has been suggested that the activation threshold of NK cells might be lower in these patients[49], which in turn might render HCV clearance more likely. It is also interesting that this epidemiological association is limited to low-dose HCV infection, because recent *in vitro* studies have shown that high concentrations of recombinant HCV E2 crosslink the tetraspanin CD81 at the surface of NK cells and inhibit their cytotoxicity and cytokine production[50,51]. Furthermore, *in vitro* studies show that NK cells from HCV-infected patients, but not from healthy control individuals, are impaired in their capacity to activate dendritic cells, owing to

ALANINE AMINOTRANSFERASE (ALT). ALT is an intracellular enzyme that transfers amino groups from L-alanine to 2-ketoglutarate or from L-glutamic acid to pyruvate. It is released into the bloodstream when hepatocytes are damaged or die. The serum ALT level (upper limit of normal is 25–40 international units per litre, depending on the laboratory) is therefore an indicator of hepatocyte injury in acute and chronic hepatitis.

EUKARYOTIC TRANSLATION INITIATION FACTOR 2α (EIF2α). A mediator of translation initiation. Phosphorylation of EIF2α by the interferon-inducible double-stranded RNA-dependent protein kinase inhibits translation and thereby indirectly inhibits viral replication.

COMPOUND GENOTYPE
A combination of two or more genotypes at loci encoding functionally related molecules.

overexpression of the receptor CD94–NKG2A (NK group 2, member A) and production of transforming growth factor-β and interleukin-10 (IL-10)[52]. It remains to be determined whether the intrahepatic concentration and the *in vivo* configuration of HCV E2 are compatible with the inhibition of NK-cell responses of infected patients.

Adaptive cellular immune responses

Patients who spontaneously recover from HBV or HCV infection typically mount vigorous multi-epitope-specific $CD4^+$ and $CD8^+$ T-cell responses that are readily detectable in blood samples. By contrast, patients with chronic hepatitis B or hepatitis C tend to have late, transient or narrowly focused T-cell responses[26,53–57].

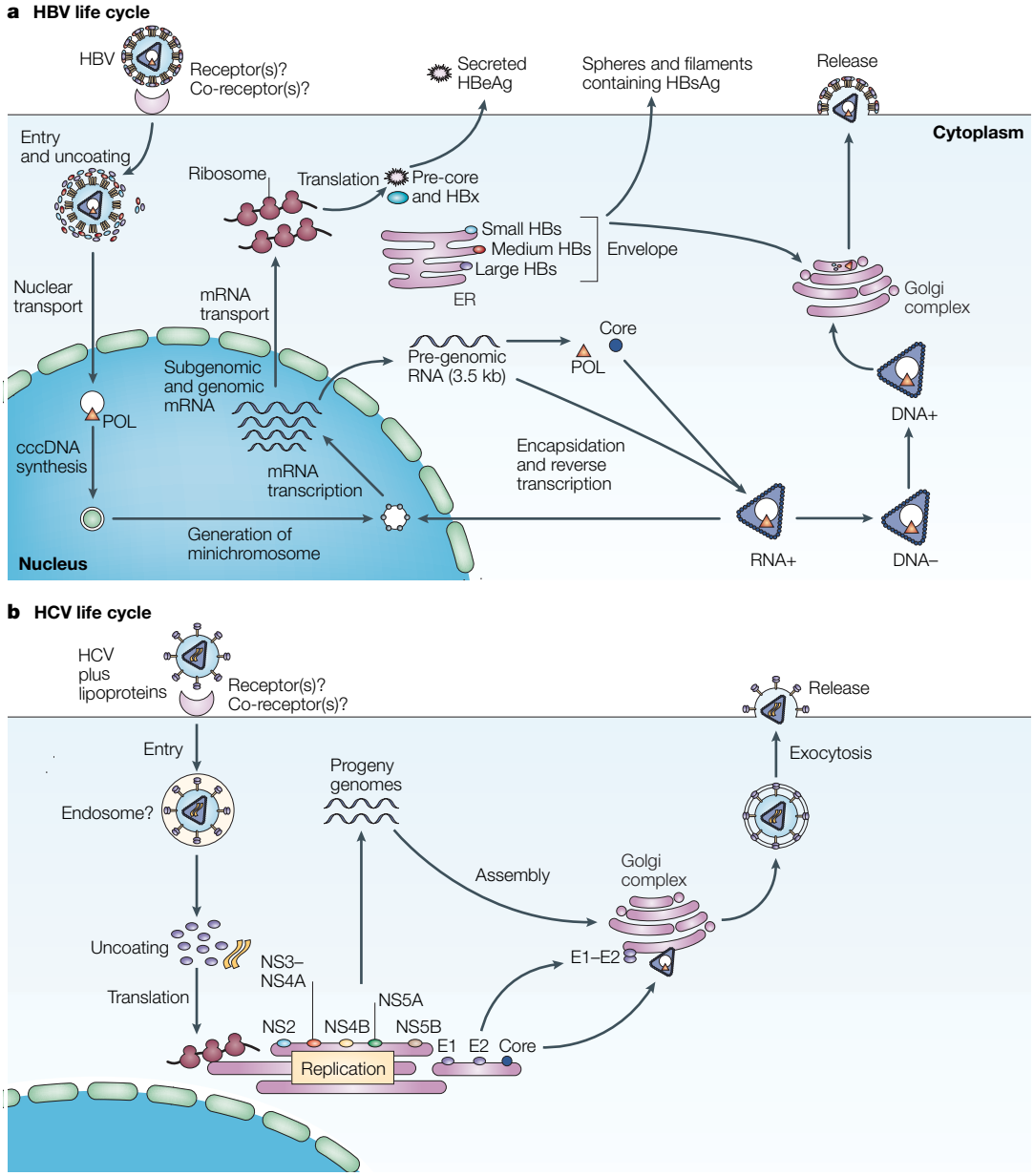

Figure 2 | **Putative life cycle of HBV and HCV. a** | After entry to the cell, hepatitis B virus (HBV) nucleocapsids transport their cargo, the genomic HBV DNA, to the nucleus, where the relaxed circular DNA is converted into covalently closed circular (ccc) DNA. The cccDNA functions as the template for the transcription of four viral RNAs (of 0.7 kilobases (kb), 2.1 kb, 2.4 kb and 3.5 kb), which are exported to the cytoplasm and used as mRNAs for the translation of the HBV proteins. The longest (pre-genomic) RNA also functions as the template for replication, which occurs within nucleocapsids in the cytoplasm[16]. Nucleocapsids are enveloped during their passage through the endoplasmic reticulum (ER) and/or Golgi complex and are then secreted from the cell. **b** | After entry to the cell, hepatitis C virus (HCV) nucleocapsids are delivered to the cytoplasm, where the viral RNA functions directly as an mRNA for translation of a long polyprotein. Replication occurs within cytoplasmic, membrane-associated replication complexes in a perinuclear membranous web[24]. Genomic RNA-containing plasmids bud through intracellular membranes into cytoplasmic vesicles, which fuse with the plasma membrane. E, envelope protein; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HBx, HBV X protein; NS, non-structural protein; POL, polymerase. Part **b** of this figure is modified from REF. 131 © 2003 with permission from Elsevier.

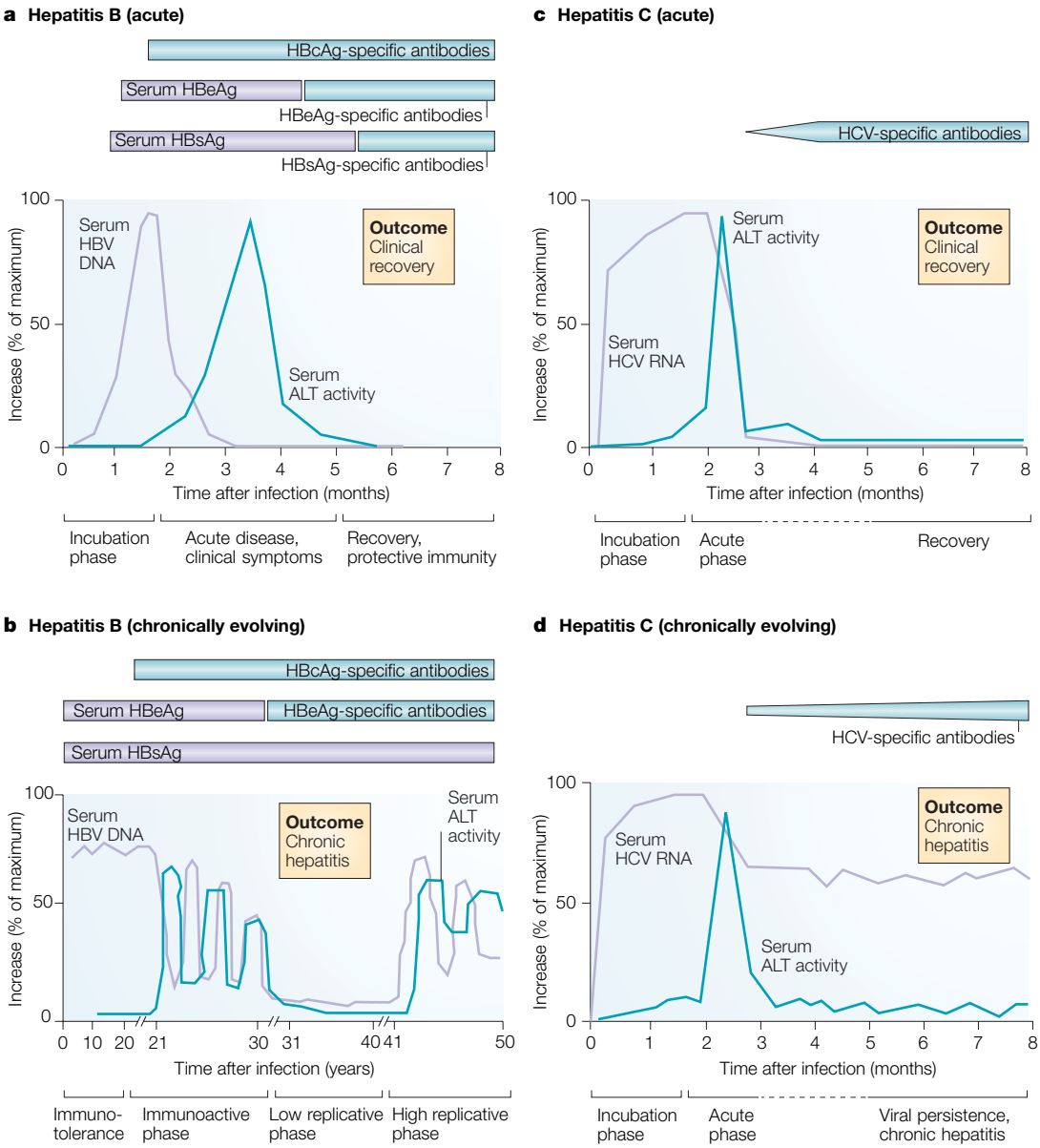

Figure 3 | **Clinical and virological course of acute infection with HBV or HCV. a** | A schematic depiction of the immune response in acute infection with hepatitis B virus (HBV) through horizontal transmission, followed by clinical recovery, is shown. After recovery, neutralizing HBV surface antigen (HBsAg)-specific antibodies and HBV-specific T cells confer lifelong, protective immunity (for further details, see main text). **b** | Chronically evolving hepatitis B results from vertical transmission. Chronic hepatitis B is most commonly seen after vertical transmission from mother to neonate. The course of disease is characterized by several phases of variable length. The immunotolerant phase is characterized by high levels of circulating HBV DNA and HBV e antigen (HBeAg) and normal alanine aminotransferase (ALT) levels, and this phase can last for decades. For unknown reasons, it can transition into an immunoactive phase, in which HBV DNA titres are lower but liver disease is markedly more severe and can progress to liver cirrhosis. Alternatively, the immunoactive phase might transition into a low replicative phase, with clearance of free HBeAg from the serum and development of HBeAg-specific antibodies. In the low replicative phase, serum HBV DNA is typically below the detection limit of hybridization assays; ALT levels also normalize, and necroinflammatory liver disease improves. The low replicative phase might last for life, but a subgroup of patients, especially those who have undergone immunosuppressive therapy, might experience recurrent high-level HBV replication and marked necroinflammatory liver disease. Mutations in the promoter region of the gene that encodes HBV core antigen (HBcAg), which are associated with increased replication, and pre-core mutations, which result in an HBeAg-negative phenotype, have been described (reviewed in REF. 1). **c** | A schematic depiction of the immune response in acute infection with hepatitis C virus (HCV), followed by clinical recovery, is shown (for further details, see main text). Note that the development of HCV-specific antibodies is variable, and clearance might occur either before the development of a measurable humoral response or even in the absence of development of a detectable antibody response. Also note that the terms 'incubation phase' and 'acute phase' are used with reference to ALT levels and not clinical symptoms. Most patients with a new HCV infection do not experience clinical symptoms. **d** | A schematic depiction of the immune response in chronically evolving hepatitis C. HCV titres decline by 2–3 logs after the ALT levels peak but then remain steady during the chronic phase of hepatitis (for further details, see main text).

Although these observations strongly indicate an association between the timing, vigour and specificity of the cellular immune response and the outcome of infection, they do not prove whether the observed T-cell responses are the cause or the consequence of viral clearance. A causal role for T-cell responses in HBV and HCV clearance was only recently proven in the chimpanzee model by the finding that *in vivo* depletion of either $CD4^+$ or $CD8^+$ T cells prevents HBV[5] and HCV[12,13] clearance and clinical recovery.

When discussing the induction and effector function of HBV- and HCV-specific T cells in viral hepatitis, several aspects that are unique to the liver should be considered. Importantly, the normal, uninfected liver maintains a largely tolerogenic environment and contains a large number of intrahepatic T cells[58]. At present, it is not clear how this tolerogenic environment, which is in part mediated by liver-specific antigen-presenting cells such as liver sinusoidal endothelial cells and Kupffer cells, changes to an inflammatory environment. It is also not clear how the pre-existing T-cell population in the liver contributes to the adaptive immune response in viral hepatitis, whether T-cell priming occurs exclusively in draining lymph nodes or whether hepatocytes can prime T cells under inflammatory conditions. These specific features of liver immunology are reviewed in REF. 58, and their impact on immune responses to hepatotropic viruses is an important area for future studies.

HBV. Non-cytolytic downregulation of viral replication seems to have a particular role in HBV infection, because most HBV DNA can be cleared from the liver and the blood of experimentally infected chimpanzees before any detectable T-cell infiltration and liver injury[4]. The cells that mediate these early antiviral effects are not readily accessible and have not yet been identified in the natural infection. However, a series of studies using transgenic mouse models showed that $CD8^+$ T cells have the capacity to non-cytolytically clear HBV from hepatocytes that replicate HBV encoded by a transgene[40]. When HBsAg-specific $CD8^+$ T cells are adoptively transferred to mice that have replication-competent copies of the HBV genome in their hepatocytes, they recognize their cognate antigen, lyse some hepatocytes and, concurrently, produce cytokines that downregulate HBV replication throughout the liver. Downregulation of HBV replication is directly linked to IFN-γ production by the adoptively transferred $CD8^+$ T cells, because it is also observed when these cells are deficient in perforin or CD95 ligand (also known as FAS ligand)[40] and when the recipient mice cannot produce endogenous IFN-γ or cannot respond to IFN-α, IFN-β or tumour-necrosis factor (TNF)[34]. Even HBV-non-specific stimuli and unrelated pathogens, such as lymphocytic choriomeningitis virus, can stimulate IFN-γ-mediated downregulation of HBV replication through activation of macrophages, NKT cells and HBV-non-specific T cells[59–61]. So, how does IFN-γ downregulate HBV replication? Single-stranded and relaxed circular replicative DNA intermediates are removed from the cytoplasm and nucleus by a post-transcriptional mechanism[62]. Specifically, HBV RNA is removed through cytokine-induced proteolytic cleavage of a nuclear ribonucleoprotein, the La autoantigen, which binds the predicted STEM LOOP of HBV RNA[63]. Removal of La autoantigen destabilizes HBV RNA and renders several endoribonuclease cleavage sites accessible to cellular RNases. In addition, IFN-γ upregulates inducible nitric-oxide synthase (iNOS), which results in the production of nitric oxide. This mechanism seems to have an essential role in this transgenic mouse model, because iNOS-deficient HBV-transgenic mice are resistant to the antiviral effects of IFN-γ and TNF[64].

An important limitation of the transgenic mouse model, however, is the absence of the cccDNA episome, the transcriptional template of HBV in the natural infection. Important supporting evidence is therefore provided by the previously mentioned prospective study in experimentally HBV-infected chimpanzees[4]. In the chimpanzee model of acute HBV infection, cccDNA disappears from the liver shortly after the other replicative intermediates, which indicates that cccDNA is also susceptible to non-cytolytic control and removal[4]. The disappearance of most of the HBV DNA from the blood and the liver is followed by increased expression of T-cell markers in the liver, maximal $CD4^+$ and $CD8^+$ T-cell responses in the blood, peak ALT levels in the blood[4,5,32] and seroconversion to HBeAg- and HBsAg-specific antibodies.

HCV. Similar to hepatitis B, the increase in serum ALT levels occurs ~8–14 weeks after HCV infection, when the intrahepatic expression of genes that encode components of the adaptive immune response (such as MHC class II molecules and chemokines) is upregulated[10,33]. Although this phase is clinically asymptomatic for most patients, it is notable that symptomatic, jaundiced patients have a higher probability of recovery than do asymptomatic patients[65]. Studies of HCV infection in chimpanzees showed that the appearance of T-cell responses and the induction of IFN-γ expression in the liver coincides precisely with a decrease in HCV RNA titres[15,27,33,66]. Whether IFN-γ exerts direct antiviral effects *in vivo* or whether it is solely a marker for other T-cell effector functions is not yet established. Direct antiviral effects would be consistent with the observation that IFN-γ inhibits replication of subgenomic and genomic HCV RNAs *in vitro*[67].

Importantly, despite the early onset of vigorous HCV replication, there seems to be a considerable delay in the appearance of HCV-specific T cells and possibly in their recruitment to the liver. Using functional assays, HCV-specific T cells have been detected in the blood of infected patients and chimpanzees 5–9 weeks after infection and in the liver of chimpanzees 6–12 weeks after infection[15,26]. Furthermore, a recent study describes that human HCV-specific T cells differ from human HBV-specific T cells in their effector functions, despite having an identical CC-chemokine receptor 7 $(CCR7)^-CD45RA^-$ EFFECTOR MEMORY CELL PHENOTYPE[68]. Whereas HBV-specific $CD8^+$ T cells express high levels

STEM LOOP
A hairpin structure that is formed by a single-stranded nucleic acid molecule when the ends of the molecule form a double helix (stem) based on complementary sequences and the central region remains single stranded and therefore forms a loop.

EFFECTOR MEMORY CELL PHENOTYPE
Phenotype of terminally differentiated T cells. These cells lack lymph-node homing receptors but express receptors that enable them to home to inflamed tissues. Effector memory cells contain perforin and can exert immediate effector functions without the need for further differentiation.

of perforin and show vigorous proliferation, IFN-γ production and cytotoxic activity on *in vitro* stimulation[68], these effector functions are reduced in HCV-specific T cells[26,57,68], and this early impairment might contribute to the lower probability of viral clearance.

Humoral immune responses

HBV-specific antibodies are indicators of specific stages of disease (FIG. 3a,b). HBcAg-specific IgM is an early marker of infection, whereas antibodies specific for HBeAg and HBsAg appear late and indicate a favourable outcome of infection (FIG. 3a). HBsAg-specific antibodies are neutralizing and mediate protective immunity. HBcAg-specific IgG and HBsAg-specific antibodies persist for life after clinical recovery. By contrast, the appearance of HCV-specific antibodies is much more variable in infected patients. No antibodies appear early after infection, and in some cases, they might not appear at all (FIG. 3c). HCV-specific antibodies are also more restricted in their isotype profile, and their end-point titre is at least 2 logs lower than that of HBV-specific antibodies[69,70]. Finally, HCV-specific antibodies are not maintained for life, as they might 'disappear' 10–20 years after recovery[30,31].

Despite these striking differences between HBV- and HCV-specific humoral immune responses, there is also some evidence that HCV-specific antibodies might influence the course of infection. Antibodies specific for the HCV envelope glycoproteins (E1 and E2) have been shown to neutralize *in vivo* infectivity of HCV in chimpanzees[71] and to modulate HCV RNA levels in vaccinated and rechallenged chimpanzees[72]. Further characterization of these humoral immune responses has long been hampered by the lack of *in vitro* models to study neutralization of virus binding and entry to the cell. Non-infectious HCV-like particles, produced using plasmids that express HCV core, E1 and E2 in the baculovirus insect-cell system, have facilitated the identification of antibodies that inhibit binding of these surrogate particles to hepatoma cell lines[73]. More recently, infectious retroviral pseudotype particles that express HCV envelope glycoproteins[21,74] allowed the identification of antibodies that neutralize the *in vitro* infectivity of these pseudotype particles. Importantly, the same immunoglobulin preparations that inhibit HCV infection of chimpanzees[75] also inhibit infection of hepatoma cell lines and primary hepatocytes by pseudotype particles, an important validation of this assay. Pseudotype-particle-neutralizing antibodies are typically strain-specific and are present at low levels during the first 6 months of HCV infection. It might take as long as 6–12 months until antibodies with increased neutralization titres and crossreactivity with E1 and/or E2 of different HCV QUASISPECIES appear[14]. Strikingly, however, the highest antibody titres are typically found in patients with established, chronic hepatitis C, and recovered patients test negative[14,21], which is consistent with the emergence of HCV escape mutants[76]. Finally, these newly developed assays might also aid in the search for the putative HCV receptor. Pseudotype-particle assays confirmed the previous observation that HCV envelope glycoproteins bind CD81 (REF. 77), because transfection of $CD81^-$ hepatoma cell lines with CD81 restores susceptibility to pseudotype-particle infection[22]. Collectively, the data indicate that CD81 is a component of the HCV receptor complex, together with other as-yet-unknown liver-specific components[22,78].

Immunological memory and protective immunity

Recovery from hepatitis B results in lasting protective immunity that is mediated by neutralizing HBsAg-specific antibodies and by HBV-specific $CD4^+$ and $CD8^+$ T cells. By contrast, recovery from hepatitis C can be followed by decline and eventual loss of HCV-specific antibodies after 10–20 years[30]. HCV-specific protective immunity has been described in some, but not all, chimpanzees that have recovered from HCV and is mediated by $CD4^+$ and $CD8^+$ T cells[9,11–13].

HBV. Although clinical recovery from acute hepatitis B is associated with lifelong protective immunity, trace amounts of virus persist in the blood of recovered patients and are controlled by cellular and humoral immune responses. Consistent with this, clinically recovered individuals who are positive for HBsAg- and HBeAg-specific antibodies and are immunosuppressed during cancer chemotherapy might experience reactivation of HBV[79]. Furthermore, organs of donors who are positive for HBsAg-specific antibody have been shown to transmit HBV to immunosuppressed transplant recipients[80]. Replicative forms of HBV are found not only in the liver but also in extrahepatic sites (reviewed in REF. 16), which indicates that immunoprivileged sites might contribute to low-level HBV persistence. Conversely, trace amounts of persisting virus might be essential for the maintenance of HBV-specific immunity in recovered individuals[81]. This hypothesis is indirectly supported by the observation that 3–5 years of effective antiviral therapy significantly reduces HBV-specific T-cell responses in patients with chronic hepatitis — in some of them, to undetectable levels[82]. It might also indicate that booster vaccinations are required to maintain vaccine-induced, HBsAg-specific humoral and cellular immune responses. This is a controversial topic because others consider that immunological memory provided by antigen-specific B and T cells[83] is sufficient for a rapid recall response, even after antibody titres decline to undetectable levels. Vaccine responses inversely correlate with age and body-mass index and are also influenced by genetic factors, such as specific HLA haplotypes, and by environmental factors, such as smoking (reviewed in REF. 84), and these factors might also influence the duration for which vaccine-induced immunity can be maintained.

HCV. As described for the immune status of patients who have spontaneously recovered from acute hepatitis B[81], virus-specific T-cell responses are also maintained by those individuals who have recovered from hepatitis C[30,57]. As shown in chimpanzees that have recovered, HCV-specific T cells not only persist in the

QUASISPECIES
A distribution of non-identical but closely related viral genomes. The entire distribution forms an organized cooperative structure, which functions as (quasi) a single unit (species).

blood but also in the liver[12], and in some cases, these cells are the only evidence of previous infection with HCV and recovery. For example, HCV-specific T cells are found in some patients who have recovered from a documented infection in the distant past and no longer have HCV-specific antibodies[30]. Whether HCV is completely cleared after recovery or whether trace amounts of HCV persist is still a matter of debate. Although HCV reactivation has not been described for patients who have recovered and are undergoing immunosuppression, HCV sequences have recently been detected in the peripheral-blood lymphocytes of clinically recovered individuals, using sensitive molecular techniques[29]. Furthermore, HCV viraemia recurred in a patient[28] and in a chimpanzee[9] after serum samples consistently tested negative for HCV RNA (using nested reverse-transcription PCR) for 4 months after normalization of ALT levels. Because loss of HCV-specific $CD4^+$ T-cell responses preceded HCV recurrence in both cases, the data indicate that HCV is usually controlled, but not completely eradicated, in the first months after clinical resolution of acute hepatitis C. Large cohort studies need to be conducted to determine how frequently trace levels of HCV RNA are detectable in patients who are long-term recovered and whether the RNA is infectious.

Whereas it is clear that recovery from hepatitis B results in lifelong protective immunity, it has long been assumed that this is not the case for recovery from hepatitis C. Multiple episodes of acute hepatitis have been reported in polytransfused THALASSAEMIC children[85], and chimpanzees that have recovered from HCV infection can be re-infected, even with homologous virus[86]. On re-infection, all chimpanzees that have been studied so far showed an attenuated course of infection, with lower HCV titres and no evidence of liver disease[9,11,12]. This is consistent with protective, albeit non-sterilizing, immunity. Rapid control of the rechallenge inoculum correlated with HCV-specific T-cell responses, whereas antibodies specific for HCV envelope glycoproteins were not detectable[9,12]. *In vivo* depletion of $CD4^+$ T cells before rechallenge resulted in chronic HCV infection[13]. *In vivo* depletion of $CD8^+$ T cells resulted in prolonged viraemia, which was controlled only when HCV-specific $CD8^+$ T cells reappeared in the liver[12].

So far, there is only limited information about whether the same type of protective immunity can also be acquired by humans[87]. In a recent epidemiological study, the risk of developing *de novo* HCV viraemia was significantly lower for intravenous drug users who had successfully cleared a previous HCV infection than for intravenous drug users who had no evidence of previous HCV infection[87]. During a follow-up period, the apparent immune protection was lost by intravenous drug users who had recovered from HCV infection but subsequently acquired HIV infection, thereby indicating a role for $CD4^+$ T cells in protective HCV-specific immunity[87]. Although these studies indicate that HCV-specific protective immunity can occur in at least some recovered patients, its incidence and duration need to be further studied.

THALASSAEMIC
An individual suffering from thalassaemia, an inherited disorder of haemoglobin metabolism that results in reduced or absent production of one or more globin chains.

Viral escape and chronic hepatitis

HBV. HBV establishes chronic hepatitis mainly by vertical transmission from HBsAg- and HBeAg-positive mothers to neonates (TABLE 1), as the immune system of neonates has not yet fully developed. Immunomodulatory effects of HBeAg might have a role in this setting, because HBeAg (which is not required for viral infection, replication and assembly) is rapidly secreted into the blood and has been shown to tolerize T cells in transgenic mice[88]. In addition, the same mechanisms that have been described to mediate downregulation of HBV replication might also facilitate viral persistence if antigen expression and presentation are reduced to levels undetectable by T cells. Another candidate mechanism, the development of viral escape mutations, seems to be more relevant for escape from vaccine-induced humoral immune responses (after active vaccination with HBsAg or passive administration of HBAg-specific antibodies) than for escape from cellular immune responses. Although HBV variants with mutations in dominant T-cell epitopes might arise during acute hepatitis B[89], they typically remain in low abundance and do not necessarily affect clinical recovery[89]. Even in chronic hepatitis B, T-cell escape mutants are not common[90], which is consistent with a weak HBV-specific T-cell response[90]. In the few chronic hepatitis B cases in which T-cell escape mutants have been observed, the T-cell response was unusually strong and narrowly focused[91] and thereby might have exerted stronger selective pressure.

HCV. In contrast to HBV, HCV mainly establishes persistent infections in adults. To explain this observation, many mechanisms have been identified in patients and chimpanzees, or have been proposed on the basis of *in vitro* studies (FIG. 4). As virus and host survival strategies, these escape mechanisms might also contribute to the attenuated, clinically asymptomatic course of new HCV infections and to the relatively slow progression of liver disease in most patients with chronic hepatitis C.

One important escape mechanism that has been directly shown is viral sequence mutations. The quasispecies nature, the comparatively high replication rate of HCV and the lack of proof-reading capacity of its polymerase contribute to rapid diversification of the viral population. The apparent delay of the adaptive cellular and humoral immune response facilitates this process so that escape mutants can be rapidly selected from the pre-existing quasispecies population when adaptive immune responses finally occur. HCV escape mutants are selected by antibodies[76,92] and T cells[93–98], as shown in studies of humans[76,94–98] and chimpanzees[92,93]. At the T-cell level, HCV escape has been reported to affect epitope processing[97,98], MHC binding[94] and T-cell-receptor stimulation[93–96].

Several additional mechanisms have been proposed to explain the described impaired effector function of HCV-specific T cells. First, a specific sequence in the HCV core protein has been shown to bind the globular domain of the receptor for the complement component C1q (which is expressed at the surface of macrophages

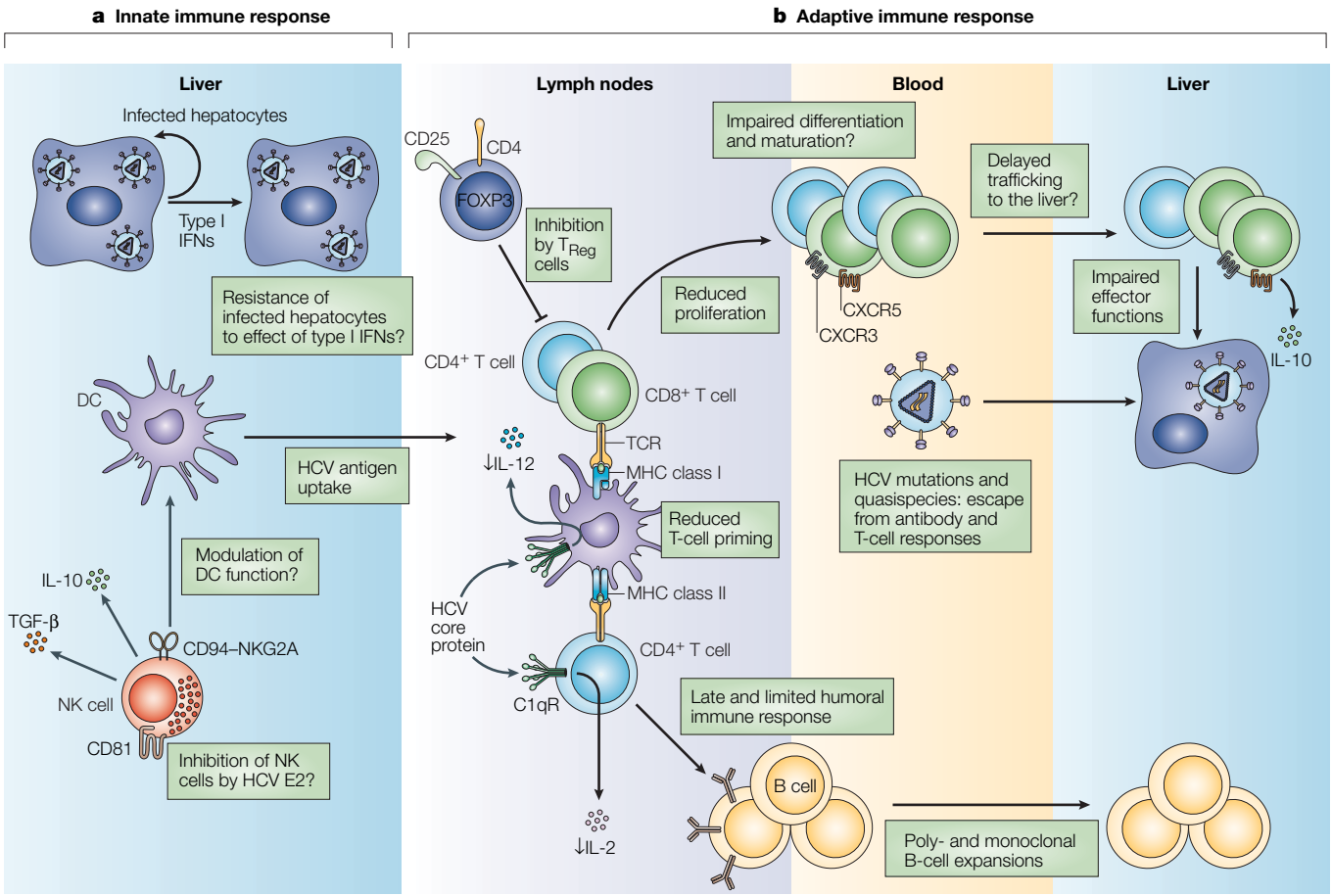

Figure 4 | **Candidate mechanisms of HCV interference with the immune system. a** | Innate immune response. Studies of experimentally hepatitis C virus (HCV)-infected chimpanzees show that intrahepatic type I interferon (IFN) responses do not correlate with the outcome of infection, indicating that HCV is not sensitive to type I IFN responses *in vivo* (for further details, see main text). In vitro studies show that natural killer (NK) cells of healthy individuals can be inhibited by high concentrations of the HCV envelope protein 2 (E2) and that NK cells of HCV-infected individuals are altered in their cytokine production and their capacity to activate dendritic cells (DCs) *in vitro*. **b** | Adaptive immune response. Viral escape from immune responses through mutations in antibody and T-cell epitopes has been shown for both HCV-infected humans[76,94–98] and chimpanzees[92,93]. Humoral immune responses appear late during infection or not at all, and they do not protect against re-infection[9,11–13,85,86]. HCV-specific T cells are less differentiated than virus-specific T cells raised to other pathogens[103], and they seem to be impaired in their effector functions[55]. Potential mechanisms include reduced T-cell priming, with a potentially altered DC function[104–107], and inhibition of macrophage and/or DC and T-cell function through binding of the HCV core protein to the receptor for the complement component C1q (C1qR)[99–101]. Furthermore, peripheral $CD4^+CD25^+$ T cells (T_{Reg} cells)[112,113] and intrahepatic interleukin-10 (IL-10)-producing $CD8^+$ T cells[111], which both have regulatory functions, have recently been detected in patients with chronic hepatitis C, and their role in the outcome of infection needs to be further analysed. Finally, despite early and high HCV titres, HCV-specific T cells are not detectable in the liver within 1 month of experimental infection of chimpanzees, which might indicate impaired trafficking to the site of infection[15]. CXCR, CXC-chemokine receptor; FOXP3, forkhead box P3; NKG2A, NK group 2, member A; TCR, T-cell receptor; TGF-β, transforming growth factor-β.

and T cells), which downregulates IL-12 production by macrophages[99] and downregulates proliferation and IL-2 and IFN-γ production by T cells[100]. Although most of these findings are from *in vitro* studies, they are supported by *in vivo* studies in which mice that were infected with recombinant HCV-core-expressing vaccinia virus showed suppressed vaccinia-virus-specific T-cell responses (IL-2 and IFN-γ production, and cytotoxicity) and higher mortality than mice that were infected with vaccinia viruses expressing either non-structural HCV proteins or an irrelevant control protein[101]. Second, recent *ex vivo* analyses of immune responses of HCV-infected patients show a correlation between suboptimal IL-2 production and an incomplete maturation and differentiation status of HCV-specific T cells[102]. Indeed, HCV-specific T cells are often less differentiated than virus-specific T cells raised to other pathogens[103]. Third, a potential impairment of dendritic-cell function has been proposed but is only described in some[104–107], but not all[108,109], patient studies, and many of these studies are limited by the use of allogeneic, not autologous, T-cell proliferation as a read-out. Fourth, host genetic factors, such as polymorphisms in cytokine gene promoters or chemokine-receptor genes[110], might contribute to the modulation of HCV-specific immune responses and, potentially, to the predominance of

IL-10-producing $CD8^+$ T cells that have been found in the liver[111]. These intrahepatic T cells show an altered effector phenotype with regulatory functions[111]. Last, $CD4^+CD25^+$ T cells with regulatory function have recently been found in the blood of patients with chronic hepatitis C[112,113]. Future studies need to address the question of whether these are naturally occurring or induced regulatory T cells and whether they are associated with a specific outcome of infection (BOX 2). Conceivably, several or all of these non-exclusive mechanisms might be operating in chronic hepatitis C and might contribute to the observed impairment of HCV-specific immune responses. The HCV-specific nature of this impairment is important, because the induction of primary and memory T cells in response to other viruses is not affected[114].

Box 2 | **The role of $CD4^+$ T cells**

Some of the earliest studies on the adaptive cellular immune response to hepatitis B virus (HBV) and hepatitis C virus (HCV) analysed the role of $CD4^+$ T cells. From these early studies, a correlation was established between a vigorous multi-specific proliferative $CD4^+$ T-cell response to recombinant viral proteins and recovery from infection with HBV or HCV[53,56]. Subsequent studies mapped $CD4^+$ T-cell epitopes — by using overlapping viral peptides and cloned $CD4^+$ T cells from peripheral blood and from liver biopsies — and these studies established the predominance of a T-helper-1 cytokine profile in both hepatitis B and hepatitis C. When MHC-class-I-binding motifs were identified, and peptide–MHC-class-I tetramers became available, $CD8^+$ T cells (the main effector cells in viral hepatitis) became an important focus of research. Recently, however, several intriguing observations have been made that re-emphasize the role of $CD4^+$ T cells and pose important questions for future studies.

First, as shown by a recent patient study[28] and by a chimpanzee study[9], HCV viraemia can recur after 4 months of apparent viral clearance from the circulation, and this recurrent viraemia is temporally related to a loss of detectable $CD4^+$ T-cell responses. The reasons for the loss of $CD4^+$ T-cell responsiveness and the requirements for the sustenance of these cells need further study.

Second, $CD4^+$ T-cell differentiation, maturation and function during the natural course of HBV and HCV infection have not yet been studied. Although the generation of MHC class II tetramers that present viral peptides has now rendered these important studies possible[129], they remain difficult, owing to the low frequency of HBV- and HCV-specific $CD4^+$ T cells in the circulation.

Third, the interplay between virus-specific $CD4^+$ and $CD8^+$ T cells is an intriguing area for further research. A recent prospective analysis of $CD4^+$ and $CD8^+$ T cells in the early phase of HCV infection showed recovery of $CD8^+$ T-cell effector function and a 5 log decrease in viraemia at precisely the time at which HCV-specific $CD4^+$ T-cell responses became detectable[26]. In another study, *in vivo* depletion of $CD4^+$ T cells from a chimpanzee that had recovered from HCV infection abrogated protective immunity on rechallenge, and viral persistence was associated with viral mutations in $CD8^+$ T-cell epitopes[13]. These studies indicate that $CD4^+$ T cells are an essential component of protective immunity. The differential contribution of the direct and indirect (through interplay with $CD8^+$ T cells, dendritic cells and B cells) antiviral effects of $CD4^+$ T cells requires further analysis.

Fourth, $CD4^+CD25^+$ T cells with regulatory functions have only recently been identified in patients with hepatitis C[112,113]. Future studies need to address the question of whether these regulatory T cells are naturally occurring or induced, whether their appearance is associated with a specific outcome of infection, and whether and how they influence the immune response in the liver.

Fifth, the role of $CD4^+$ T cells in the generation of humoral immune responses needs to be further analysed. Although the production of HBV e antigen (HBeAg)-specific antibodies is strictly dependent on $CD4^+$ T cells and although the production of HBV core antigen (HBcAg)-specific antibodies can occur both in a T-cell-dependent and -independent manner[130], the role of $CD4^+$ T cells in the generation of HCV-specific antibodies is not yet clear.

Last, there is still a need to map T-cell epitopes, particularly those that are restricted by HLA alleles commonly found in individuals from Asia and Africa.

Immunological aspects of chronic hepatitis

Although both HBV and HCV infection can result in chronic hepatitis, cirrhosis and hepatocellular carcinoma, several immunological differences between chronic hepatitis B and hepatitis C should be noted, as they might be relevant for the development of immunomodulatory therapies and therapeutic vaccines.

Significant changes in viral titre and alternating periods of immunotolerance and severe immunopathology have been described for patients with chronic hepatitis B. Hepatitis B 'flares' are temporally related to increased serum IL-12 levels[115] and increased $CD4^+$ T-cell responses to HBV nucleocapsid antigens[115,116]. Each year, 2% of patients with chronic hepatitis B spontaneously clear free HBsAg and develop neutralizing HBsAg-specific antibodies[1], and HBV-specific T-cell responses have been detected in the blood just before seroconversion[116]. Accordingly, it has been shown that effective therapeutic reduction of HBV titres results in a transient restoration of HBV-specific $CD4^+$ and $CD8^+$ T-cell responses in the blood of patients with chronic hepatitis B[117–119]. Collectively, these findings indicate that immune-mediated clearance mechanisms can be spontaneously activated or induced, even in chronic hepatitis B. Although this might be advantageous for therapeutic induction of such responses, it might also increase the risk of immunopathology. By contrast, HCV RNA titres tend to remain stable for decades in patients with chronic hepatitis C (FIG. 3d), and there seems to be no spontaneous viral clearance.

Another aspect of immunological interest is the pathogenesis of liver disease in HBV and HCV infections. In both HBV and HCV infection, the pathogenesis of chronic hepatitis and cirrhosis is thought to be immune mediated. It has therefore long been assumed that chronic HBV carriers without marked liver disease have fewer or no HBV-specific T cells. Recent studies, however, showed functional, tetramer-positive $CD8^+$ T cells in the blood and the liver of these patients[120]. Furthermore, the number of intrahepatic, HBV-specific, tetramer-positive T cells did not differ between HBeAg-negative patients with normal ALT levels and HBeAg-positive patients with increased ALT levels, even though the intrahepatic cellular infiltrate was greater in the latter group[120]. These findings indicate a possible differential contribution of HBV-specific and HBV-non-specific bystander lymphocytes to the pathogenesis of liver disease in hepatitis B. In the absence of a small animal model of chronic hepatitis, this interesting topic is difficult to study. Studies using the transgenic mouse model of acute hepatitis B, however, provide several interesting insights. Adoptive transfer of HBsAg-specific $CD8^+$ T cells to transgenic mice that have replication-competent copies of HBV in their hepatocytes results in the rapid recruitment of HBV-non-specific bystander lymphocytes[121–123]. Whereas the adoptively transferred HBsAg-specific T cells lyse a

relatively small number of hepatocytes by direct cell–cell contact[121] and downregulate HBV replication non-cytolytically throughout the liver by secretion of cytokines, acute liver injury becomes most evident when non-specific, chemokine-mediated infiltration of neutrophils, NK cells and activated bystander lymphocytes occurs[121,122]. Interestingly, recruitment of antigen-non-specific mononuclear cells can be reduced and liver injury can be prevented by inactivation of macrophages, neutralization of chemokines or blocking of neutrophil-derived matrix metalloproteinases[123]. Remarkably, this inhibition of the non-specific amplification does not affect the non-cytolytic downregulation of HBV replication by HBV-specific $CD8^+$ T cells[122,123]. Whereas these mechanisms show an intriguing role for antigen-non-specific responses in acute liver injury, a small animal model of chronic hepatitis needs to be developed to determine whether similar mechanisms contribute to chronic liver injury. If so, these mechanisms might be inhibited therapeutically to prevent long-term complications of chronic inflammatory liver disease, such as cirrhosis, and to decrease the risk of development of hepatocellular carcinoma.

In this respect, it is notable that hepatocellular carcinoma might develop in the absence of cirrhosis in patients with chronic hepatitis B, but it almost always develops on the background of liver cirrhosis in patients with hepatitis C. This observation indicates a differential contribution of viral and host factors to hepatocarcinogenesis in hepatitis B and hepatitis C, which is reviewed in REFS 16,124.

Finally, both hepatitis B and hepatitis C are also associated with extrahepatic manifestations of disease. In both infections, extrahepatic manifestations can be mediated by virus-specific immune-complex injury, and they include arthritis, vasculitis and glomerulonephritis (reviewed in REFS 124,125). In addition, mono- and polyclonal B-cell expansions have been observed in chronic hepatitis C and can evolve into mixed cryoglobulinaemia[125] and into B-cell malignancies, such as non-Hodgkin's lymphoma[126,127]. HCV-induced mutations in proto-oncogenes have been implicated in this process[127,128].

Concluding remarks

The host immune response has a unique role in viral hepatitis because it contributes not only to viral control, clinical recovery and protective immunity but also to chronic hepatitis and liver cirrhosis. Although HBV and HCV are both hepatotropic viruses that induce acute and chronic liver disease, they differ markedly in the way that they interact with the host immune system. The most notable manifestation of these different patterns of virus–host interaction is that HBV is controlled by most newly infected adults (and establishes chronic infection mainly by infecting neonates), whereas HCV readily establishes chronic infection in adults. As outlined here, multiple factors — such as genome composition and replication strategy, induction of, and sensitivity to, innate immune responses, as well as mechanisms of escape from adaptive immune responses — have a role in this process. It is to be hoped that recent advances in our understanding of the immunological mechanisms of virus–host interactions, protective immunity and disease pathogenesis will help us to develop vaccines against HCV infection and immunotherapies that cure patients with persistent HBV and/or HCV infection.

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Competing interests statement
The authors declare no competing financial interests.

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