Immunity
Review

Immune Responses to HCV and Other Hepatitis Viruses

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Five human hepatitis viruses cause most of the acute and chronic liver disease worldwide. Over the past 25 years, hepatitis C virus (HCV) in particular has received much interest because of its ability to persist in most immunocompetent adults and because of the lack of a protective vaccine. Here we examine innate and adaptive immune responses to HCV infection. Although HCV activates an innate immune response, it employs an elaborate set of mechanisms to evade interferon (IFN)-based antiviral immunity. By comparing innate and adaptive immune responses to HCV with those to hepatitis A and B viruses, we suggest that prolonged innate immune activation by HCV impairs the development of successful adaptive immune responses. Comparative immunology provides insights into the maintenance of immune protection. We conclude by discussing prospects for an HCV vaccine and future research needs for the hepatitis viruses.

Introduction

Although epidemics of jaundice, most likely caused by viral hepatitis, date back thousands of years to ancient China (Fonseca, 2010), the five viruses (named hepatitis A to E viruses) that are responsible for the majority of the world's cases of acute and chronic hepatitis were discovered only in the past 60 years (Table 1). Combined, they cause a wide spectrum of disease ranging from inapparent to fulminant hepatitis in acute infection and from mild necroinflammatory liver disease to cirrhosis and hepatocellular carcinoma in chronic infection. They are also responsible for the majority of liver transplantations worldwide.

Hepatitis B virus (HBV), the first such described virus, is responsible for about 350 million chronic infections worldwide, mostly resulting from vertical transmission from mother to child (Chisari et al., 2010). HBV is a small, enveloped DNA virus that establishes a minigenome, the covalently closed circular DNA (cccDNA), as its transcriptional template in host cells (Levrero et al., 2009). In contrast to infection early in life, infection during adulthood usually results in acute self-resolving hepatitis and protective immunity (Chisari et al., 2010). Hepatitis D virus (HDV) is an enveloped, negative sense, single-stranded, closed circular RNA virus. HDV requires HBV to propagate, and infection with both viruses typically results in severe liver pathology (Taylor, 2012). Hepatitis A virus (HAV) and hepatitis E virus (HEV) are positive-stranded nonenveloped RNA viruses transmitted via the fecal-oral route and typically cleared after acute infection of immunocompetent individuals. A high incidence of severe HAV infections is observed among nonvaccinated persons who encounter the infection late in life and many HEV infections are fatal in pregnant women (Hoofnagle et al., 2012; Qu and Lemon, 2010). Hepatitis C virus (HCV) is also a positive-stranded RNA virus, but it is parenterally transmitted and establishes a high percentage of chronic infections upon transmission between adults. About 170 million people worldwide are infected and at risk of developing liver cirrhosis and hepatocellular carcinoma (Rehermann, 2009). Although great progress has been made in the development of antiviral therapies for HCV (Heim, 2013; Scheel and Rice, 2013), HCV remains the sole hepatitis virus for which a vaccine is not yet available. A complicating factor for the development of an HCV vaccine is the absence of antibody-based sterilizing immunity against reinfection (Liang, 2013).

This review describes our current knowledge of innate and adaptive immune responses to HCV. Studies of HCV's elaborate mechanisms to prevent and counteract the host innate and adaptive immune responses have yielded many discoveries that range from genetic variants affecting spontaneous and interferon-α (IFN-α)-based viral clearance to pathways of IFN and interferon-stimulated gene (ISG) induction and evasion, sensing of viral RNA by noninfected nonparenchymal cells, and conditions for success and failure of adaptive immune responses and protective immunity. Comparative aspects of HAV and HBV immunology are highlighted because they provide unique insights into the link between innate and adaptive responses and the maintenance of immune memory. The latter may be relevant for the development of a prophylactic T cell-based vaccine against HCV. Future research needs for hepatitis A, B, and C virus infection and the immunologically barely studied hepatitis D and E virus infections are described at the end of the review.

Innate Immune Responses to HCV

Many patients are diagnosed with HCV infection after decades of chronic hepatitis. Despite high viral titers, HCV proteins are expressed at such low amounts that immunohistochemistry is not adequate to assess the proportion of infected hepatocytes. Advanced techniques such as 2-photon microscopy (Liang et al., 2009) and in situ hybridization with large sets of HCV isolate-specific probes (Wieland et al., 2013) estimate that up to 30%–50% of hepatocytes are infected in patients with chronic hepatitis C. Hepatocytes are infected by blood-borne HCV through a complex series of events that includes binding of the virus to several low-affinity attachment receptors in addition to the tetraspanin CD81, the scavenger receptor class B type I (SR-B1), the tight junction proteins occludin (OCLN) and claudin (CLDN1), and the cholesterol absorption receptor Niemann-Pick C1-like 1 (NPC1L1) (Lindenbach and Rice, 2013). In addition, cell-to-cell transmission of infectivity has been demonstrated in hepatoma cell cultures in the presence of neutralizing antibodies (Ramakrishnaiah et al., 2013; Timpe et al., 2008). This is consistent with

Table 1. The Hepatitis Viruses

	HAV	HBV	HCV	HDV	HEV
Discovery (year)	1973	1965 (HBsAg)	1989	1977 (Delta antigen)	1983 (virus particle)
		1970 (HBV particle)		1986 (HDV cloned)	1990 (HEV cloned)
Genome	+ssRNA	partially dsDNA	+ssRNA	−ssRNA	+ssRNA
Virus structure	28 nm; nonenveloped nucleocapsid	42 nm; enveloped nucleocapsid	50 nm; enveloped nucleocapsid	40 nm; enveloped nucleocapsid	27–34 nm; nonenveloped nucleocapsid
Classification	Hepatovirus genus; Picornaviridae family	Orthohepadnavirus genus; Hepadnaviridae family	Hepacivirus genus; Flaviviridae family	Deltavirus genus	Hepevirus genus; Hepeviridae family
Genotype	3 major genotypes; 6 subtypes	8 genotypes (8% intergroup divergence)	6 major genotypes; more than 50 subtypes; quasispecies in each infected patient	8 genotypes	4 genotypes; 24 subtypes
Mutation rate	high (1/1,000–1/6,000 bases/year)	low (1/100,000 bases/year)	high (1/1,000 bases/year)	high (1/300–1/3,000 bases/year)	high (1/700 bases/year)
Viral half-life time	unknown	2–3 days	3 hr	unknown	unknown
Virion production	unknown	10^{10}–10^{12} virions/day	10^{12} virions/day	unknown	unknown
Worldwide prevalence	1.5 million people	350 million people	170 million people	20 million people	14 million people
Transmission	enteral, fecal-oral	parenteral	parenteral	parenteral	enteral, fecal-oral; infrequent: parenteral
Disease outcome	acute, self-limited	mostly acute, self-limited if infection occurs during adulthood; mostly chronic after neonatal infection	acute, 60%–80% develop chronic infection	depends on HBV infection; 2% of HBV/HDV coinfections and 90% of HDV superinfections persist	acute, self-limited; chronic infection mainly in immunocompromised patients
		15%–25% of patients with chronic hepatitis B die from liver failure or liver carcinoma	1%–5% of patients with chronic hepatitis C die from liver failure or liver carcinoma	70%–80% of patients with chronic hepatitis D develop cirrhosis; 60% of these die within 10 years	57,000 death/year in worldwide
Vaccine	yes (inactivated or live attenuated); vaccination prevents infection for more than 25 years	yes (recombinant HBsAg); vaccination of neonates prevents persistent infection	no (not available)	vaccine against HBV protects against HDV infection	yes (approved in China)
Treatment of persistent infection	not applicable	IFN-α-based therapy achieves seroconversion in a minority of patients; nucleos(t)ide analogs suppress but do not eradication of HBV	pegylated IFN-α, ribavirin, direct acting antivirals; HCV clearance in 45%–80% of individuals depending on HCV genotype	high doses of IFN-α-based therapy effective in only about 20% of patients; HDV relapses in most cases after cessation of treatment	not applicable

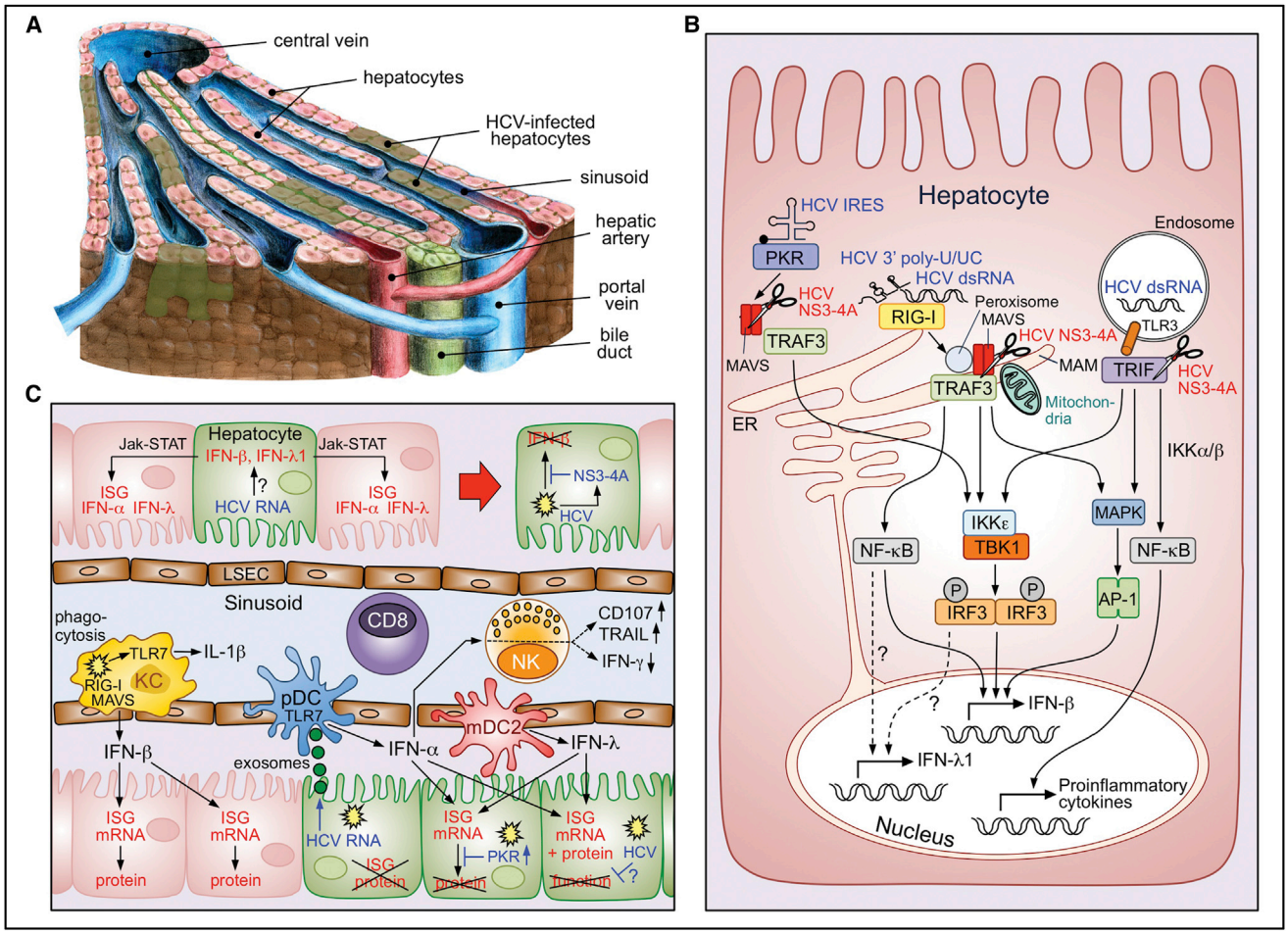

Figure 1. HCV Induces and Evades Innate Immune Responses in the Liver

(A) HCV-infected hepatocytes form clusters (indicated in green) in the infected liver (Wieland et al., 2013), consistent with the cell-to-cell transmission observed in hepatoma cultures (Ramakrishnaiah et al., 2013; Timpe et al., 2008).

(B) Specific HCV RNA structures are recognized by PKR, RIG-I, and TLR3 in cell culture experiments. PKR and RIG-I signals are transmitted via MAVS, and TLR3 signals are transmitted via TRIF. Downstream activation of kinases, IRF-3-dependent induction of IFN-β, and NF-κB-dependent induction of proinflammatory cytokines such as TNF-α and CXCL10 is abrogated in the presence of HCV NS3-4A, which cleaves both MAVS (Foy et al., 2003) and TRIF (Li et al., 2005). The induction of *IFNL1* transcription is indicated by a dotted line because this pathway has been demonstrated in other models (Osterlund et al., 2007) and not yet been confirmed for HCV infection. The secreted interferons bind to their respective receptors, which via activation of the Jak-STAT pathway amplifies the IFN response and induces a large set of ISGs (not shown). Abbreviations are as follows: IRES, internal ribosomal entry site; dsRNA, double-stranded RNA; PKR, RNA-dependent protein kinase R; RIG-I, retinoic acid-inducible gene 1; TLR3, toll-like receptor 3; MAM, mitochondria-associated endoplasmic reticulum membrane; MAVS, mitochondrial antiviral signaling protein; TBK1, TANK-binding kinase 1; TRIF, Toll/IL-1 receptor domain-containing adaptor inducing IFN-β; IRF-3, IFN-regulatory factor 3.

(C) HCV persists in the presence of ISGs. IFN-β production is blocked in the upper right hepatocytes because of intracellular expression of HCV NS3-4A. Whether individual hepatocytes transiently express IFN-β and potentially IFN-λ1 in response to HCV RNA, e.g., in the context of an abortive intracellular infection or prior to accumulating sufficient NS3-4A as indicated in the upper left hepatocyte, is not known at present. Nonparenchymal cells, e.g., Kupffer cells, pDCs, and BDCA3$^+$ mDC2s may contribute to the production of ISG-inducing IFNs. The induced IFNs bind to their respective receptors, activate the Jak-STAT pathway, and induce ISG mRNA and proteins in HCV-uninfected hepatocytes (lower part of the figure). Although ISG mRNAs are induced in HCV-infected hepatocytes (indicated in green), HCV may persist by phosphorylating and activating PKR. PKR blocks cap-dependent translation of host proteins but not IRES-dependent translation of HCV proteins (see text for details). Continuous exposure to IFN-α increases STAT1 expression in NK cells and results in increased pSTAT1-dependent cytotoxicity (CD107a expression and TRAIL production) and decreased pSTAT4-dependent IFN-γ production (see text for details). Abbreviations are as follows: KC, Kupffer cell; pDC, plasmacytoid dendritic cell; mDC2, myeloid dendritic cell; NK, natural killer cell; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

the finding that HCV-infected hepatocytes are arranged in clusters in the infected liver (Figure 1A; Wieland et al., 2013).

In infected hepatocytes, the HCV RNA genome is translated into a single protein that is cotranslationally and posttranslationally processed by cellular and viral proteases into structural (C, E1, E2, p7) and nonstructural (NS2–NS5B) proteins. The nonstructural proteins become a part of a perinuclear membrane-associated replication complex in which progeny positive-strand RNA molecules are transcribed via negative-strand RNA intermediates (Lindenbach and Rice, 2013).

Analysis of the full HCV life cycle became possible only in 2005 when the highly replicative HCV JFH-1 strain was cloned and used to establish infection in human hepatoma cells (Lindenbach et al., 2006; Wakita et al., 2005; Zhong et al., 2005). Other

systems to dissect intracellular innate immune responses include hepatoma cells transfected with HCV RNA or subgenomic HCV replicons (Lohmann and Bartenschlager, 2013). In these models, several HCV RNA structures are recognized by cytosolic pattern recognition receptors (Figure 1B). The HCV internal ribosomal entry site (IRES) is recognized by RNA-dependent protein kinase R (PKR) whereas RIG-I seems to be important for recognition of the HCV 3′ poly-U/UC sequence, the 5′ triphospate of the uncapped HCV RNA, and short double-stranded RNA regions (reviewed in Horner and Gale, 2013). Longer double-stranded HCV RNA intermediates activate endosomal toll-like receptor 3 (TLR3) but in infected hepatoma cells that ectopically express this receptor, this occurs after viral recognition by PKR and RIG-I (Li et al., 2012; Wang et al., 2009). Upon activation, PKR and RIG-I signals are transmitted via mitochondrial antiviral signaling protein (MAVS, also called VISA, IPS-1, and CARDIF), whereas TLR3 signals are transmitted via the adaptor molecule Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) (reviewed in Horner and Gale, 2013). These signaling cascades can lead to the synthesis of IFN-β via activation of TANK-binding kinase 1 (TBK1) and phosphorylation, dimerization, and nuclear translocation of IFN-regulatory factor 3 (IRF-3). In addition, synthesis of proinflammatory cytokines such as TNF-α and CXCL10 can result from activation of the canonical IκB kinase complex and nuclear translocation of NF-κB. However, HCV escapes from such innate responses by NS3-4A serine protease-mediated cleavage of MAVS (Foy et al., 2003) and TRIF (Li et al., 2005), which blocks IFN-β and ISG induction in infected hepatoma cells (Cheng et al., 2006). This is supported by translational studies that demonstrate the presence of cleaved MAVS in the liver biopsies of HCV- but not HBV-infected patients (Bellecave et al., 2010). MAVS cleavage is specifically detected in HCV NS3-expressing hepatocytes (Loo et al., 2006) along with the cytoplasmic (i.e., inactive) form of IRF-3 (Lau et al., 2008). Whether IFN production is completely blocked in HCV-infected hepatocytes or whether individual hepatocytes transiently express type I and III IFNs in situ in response to HCV RNA, e.g., in the context of an abortive intracellular infection or prior to accumulating sufficient NS3-4A (Figure 1C), is unknown.

It is established, however, that noninfected nonparenchymal cells are an alternative source of type I and III IFNs during HCV infection (Figure 1C). Kupffer cells, the dominant macrophage population in the liver sinusoids, express IFN-β protein in liver biopsies of HCV-infected patients (Lau et al., 2013). This is consistent with the induction of IFN-β in a human macrophage cell line after in vitro exposure to HCV (Lau et al., 2013). Phagocytic uptake of HCV RNA also triggers an inflammatory IL-1β response via TLR7-mediated inflammasome activation in these cells (Negash et al., 2013). Plasmacytoid dendritic cells (pDCs) constitute a second nonparenchymal cell population that may contribute to the intrahepatic IFN response. Although pDC cultures do not respond directly to HCV (Shiina and Rehermann, 2008), they have been shown to take up HCV RNA via exosomes from hepatoma cells that harbor subgenomic HCV replicons (Takahashi et al., 2010). This process requires direct cell-to-cell contact and results in the activation of TLR7 within endosomes and in production of IFN-α by pDCs (Dreux et al., 2012). However, this mechanism still needs to be confirmed in the infected liver.

Finally, IFN-λs may contribute to ISG induction. IFN-λ1 in particular has been detected at high concentrations in sera of chimpanzees with acute HCV infection (Park et al., 2012a) and in patients with chronic HCV infection (Thomas et al., 2012). Similar to IFN-α and IFN-β, IFN-λs can be produced by nonparenchymal cells and thereby bypass an HCV-induced attenuation of type I IFN production in HCV-infected hepatocytes. For example, in coculture experiments, human BDCA3$^+$ myeloid DCs (mDC2s), which are rare in the circulation but enriched in the liver, recognize HCV-infected hepatoma cells in a TLR3- and TRIF-mediated manner and produce IFN-λs (Figure 1C; Zhang et al., 2013). Thus, it is conceivable that IFN-α, IFN-β, and IFN-λ production by nonparenchymal cells contribute to ISG induction in the HCV-infected liver by stimulating the receptor for IFN-α and IFN-β and the receptor for IFN-λ and the downstream Jak-STAT pathway in hepatocytes. The complete characterization of induced the ISGs and their antiviral effect and mechanism of action is an area of intense investigation (Metz et al., 2013; Schoggins et al., 2011).

How does HCV persist in the presence of high ISG expression? Based on experiments with overexpressed HCV proteins, in either transfected cells or transgenic mice (reviewed in Rehermann, 2009), interference with Jak-STAT signaling was initially proposed as an HCV escape strategy. However, Jak-STAT signaling is not impaired in HCV-infected cells with lower, supposedly more physiologic, amounts of HCV proteins (Cheng et al., 2006). Furthermore, HCV RNA and ISG mRNA are simultaneously detected in hepatocytes of patients with chronic HCV infection (Wieland et al., 2013). A possible alternative explanation is a posttranscriptional block in ISG expression resulting from HCV-induced phosphorylation of PKR. PKR is an ISG that inhibits the elongation initiation factor 2α and thereby decreases the cap-dependent translation of cellular mRNAs as shown in HCV-infected hepatoma cells (Garaigorta and Chisari, 2009). Translation of HCV proteins is less affected in this setting because it occurs in an IRES-dependent manner (Figure 1C; Garaigorta and Chisari, 2009). Finally, HCV may interfere directly with the function of individual ISGs (Figure 1C), but the complexity and redundancy of the ISG system would require an arsenal of target-specific interference strategies. Collectively, these findings suggest that clusters of HCV-infected hepatocytes are surrounded by rings of noninfected hepatocytes that express antiviral proteins encoded by ISGs. Advanced imaging techniques may be used to examine whether such structures are locked in place or whether they move through the liver, perhaps influenced by the differentiation status of individual hepatocytes or regeneration of the liver itself.

Chronic Innate Immune Activation Negatively Predicts the Response to IFN-α-Based Therapy

Continued ISG expression in the chronically HCV-infected liver has both clinical and immunological consequences. It is the strongest negative predictor of a response to IFN-α-based therapy, the standard treatment for the past 25 years (Heim, 2013). High intrahepatic ISG expression prior to therapy is associated with a minor increase in expression during IFN-α-based therapy and lack of a virological response (Dill et al., 2011). Likewise, the amount of nuclear pSTAT1 prior to treatment is higher in hepatocytes of nonresponders to IFN-α-based therapy than in

those of responders, which suggests that the preactivated IFN response is refractory to further stimulation (Sarasin-Filipowicz et al., 2008). This correlation extends to innate immune cells that respond to IFN-α. NK cells of patients with chronic HCV infection display higher amounts of STAT1 than do those of healthy controls (Miyagi et al., 2010). This is accompanied by increased NK cell cytotoxicity and decreased IFN-γ production (Ahlenstiel et al., 2010; Oliviero et al., 2009). Based on a mechanism first described in mice infected with lymphocytic choriomeningitis virus (LCMV), this reflects an IFN-α signature because increased STAT1 expression results in preferential phosphorylation of STAT1 over STAT4 and an increase in pSTAT1-dependent NK cell cytotoxicity over pSTAT4-dependent IFN-γ production (Miyagi et al., 2007). The continued IFN-α-mediated stimulation of NK cell cytotoxicity in the absence of viral clearance may fulfill an immunoregulatory role rather than antiviral role as discussed in a recent review (Rehermann, 2013). Consistent with what has been reported for intrahepatic ISG expression, there is a negative correlation between the pretreatment activation status of NK cells and the relative increase in their activation status during IFN-α-based therapy, which predicts the subsequent virological treatment response (De Maria et al., 2007). Furthermore, NK cells from patients with rapid HCV RNA decline upon treatment initiation exhibit maximal in vivo pSTAT1 induction in response to IFN-α-based therapy, whereas NK cells of patients with slow HCV RNA decline exhibit lower in vivo pSTAT1 induction (Edlich et al., 2012). This is consistent with greater increase in NK cell cytotoxicity during IFN-α-based therapy in treatment responders than in nonresponders (Ahlenstiel et al., 2011).

Recently identified genetic variants on chromosome 19 within or near the *IFNL3* gene are also associated with spontaneous and treatment-induced outcome of HCV infection and add to the predictive value of pretreatment expression of ISGs (Ge et al., 2009; Tanaka et al., 2009; Thomas et al., 2009). The favorable rs12979860 CC-variant, located 3 kb upstream of the *IFNL3* gene, is more prevalent in Europeans and Asians than in African Americans and therefore tracks with the differential treatment response rates of these populations. However, the mechanisms for the genetic basis of HCV clearance in the context of rs12979860 and the related rs8103142, rs4803217, and ss469415590 SNPs in the same haplotype block are not understood. The rs8103142 SNP is located in the *IFNL3* coding region but does not change the functional properties of the IFNL3 protein (Urban et al., 2010). The rs4803217 SNP is located in the 3′ untranslated region of the *IFNL3*, and its T-variant may decrease the stability of the *IFNL3* mRNA via posttranscriptional regulation by HCV-induced miRNAs based on in vitro experiments (McFarland et al., 2013). However, there is no consistent demonstration that differential *IFNL3* mRNA or IFN-λ3 protein expression in HCV-infected patients are associated with divergent outcomes of HCV infection (Dill et al., 2011; Honda et al., 2010; Langhans et al., 2011; Urban et al., 2010). The ss469415590 SNP is located between *IFNL3* and *IFNL2* and improves the prediction of treatment outcome for African Americans. Its ΔG dinucleotide variant creates an open-reading frame in *IFNL4* (Prokunina-Olsson et al., 2013). Overexpression of an IFN-λ4 fusion protein induces ISGs and antiviral activity in transfected hepatoma cells but an infection-induced secreted protein has not yet been detected. Thus, the mechanisms that explain the genetic determinants of HCV clearance are not understood at present, and ISG expression remains an independent negative predictor of the response to IFN-based therapy.

Adaptive Immune Responses to HCV

A unique feature of HCV infection is the late appearance of adaptive immune responses, which typically are detectable no earlier than 8–12 weeks after infection in both self-limited (Figure 2A) and chronically evolving (Figure 2B) hepatitis C. In the following sections, we describe HCV-specific responses and compare them to HAV- and HBV-specific responses, leading to the hypothesis that prolonged ISG activation interferes with the development of protective immunity.

The small percentage of patients who clear HCV typically mount broad $CD4^+$ T cell responses in the acute phase of HCV infection with better T cell proliferation and IL-2, IFN-γ, and TNF-α production than patients who develop chronic infection (Figure 2C; Diepolder et al., 1995; Missale et al., 1996; Smyk-Pearson et al., 2008; Urbani et al., 2006). In particular, the differential expansion of $CD161^{hi}CCR6^+CD26^+$ $CD4^+$ T cells that express IL-17A and IL-21 along with Th17 cell lineage-specific transcription factors appears to correlate with the outcome of infection (Kared et al., 2013). IL-17A plasma concentrations are higher in the acute phase of hepatitis in patients with self-limited infection than in patients with chronically evolving HCV infection. This is followed by differential IL-21 concentration several weeks later (Kared et al., 2013). If broadly directed $CD4^+$ T cell responses are detected in acutely infected patients who later develop chronic hepatitis, these T cells undergo rapid exhaustion with sequential loss of IL-2 production, proliferation, and IFN-γ production (Gerlach et al., 1999; Semmo et al., 2005) and increased expression of the inhibitory receptors T cell immunoglobulin domain and mucin domain 3 (TIM-3), programmed death 1 (PD-1), and cytotoxic T lymphocyte antigen 4 (CTLA-4) (Raziorrouh et al., 2011). An expansion of galectin-9 (Gal-9) expressing regulatory T (Treg) cells and increased Gal-9 plasma concentrations in these patients has been reported (Kared et al., 2013), and it has been proposed that binding of Gal-9 to Tim-3 inhibits IL-21-producing HCV-specific Th17 cells (Figure 2C).

In contrast to $CD4^+$ T cells, HCV-specific $CD8^+$ T cells are typically "stunned" in all patients in the early acute phase of HCV infection as evidenced by impaired proliferation, IFN-γ production, and cytotoxicity (Lechner et al., 2000; Thimme et al., 2001; Urbani et al., 2006) and increased cell surface expression of the PD-1 molecule (Figure 2C; Kasprowicz et al., 2008). Initiation of IFN-α-based therapy in the acute infection results in a rapid decay of $CD8^+$ T cell responses (Rahman et al., 2004), suggesting that most HCV-specific $CD8^+$ T cells are short lived, antigen-dependent effector cells rather than self-sustaining memory T cells in this phase. However, PD-1 expression on HCV-specific $CD8^+$ T cells can be transient in acute hepatitis C and, along with CD38 expression, can reflect $CD8^+$ T cell activation rather than exhaustion in this phase of the response (Kasprowicz et al., 2008; Shin et al., 2013). If HCV infection is self-limited, the HCV-specific $CD8^+$ T cell population loses expression of PD-1 and gains expression of the antiapoptotic molecule Bcl-2 and the IL-7 receptor CD127 (Shin et al., 2013; Thimme et al., 2001; Urbani et al., 2006). HCV-specific $CD8^+$

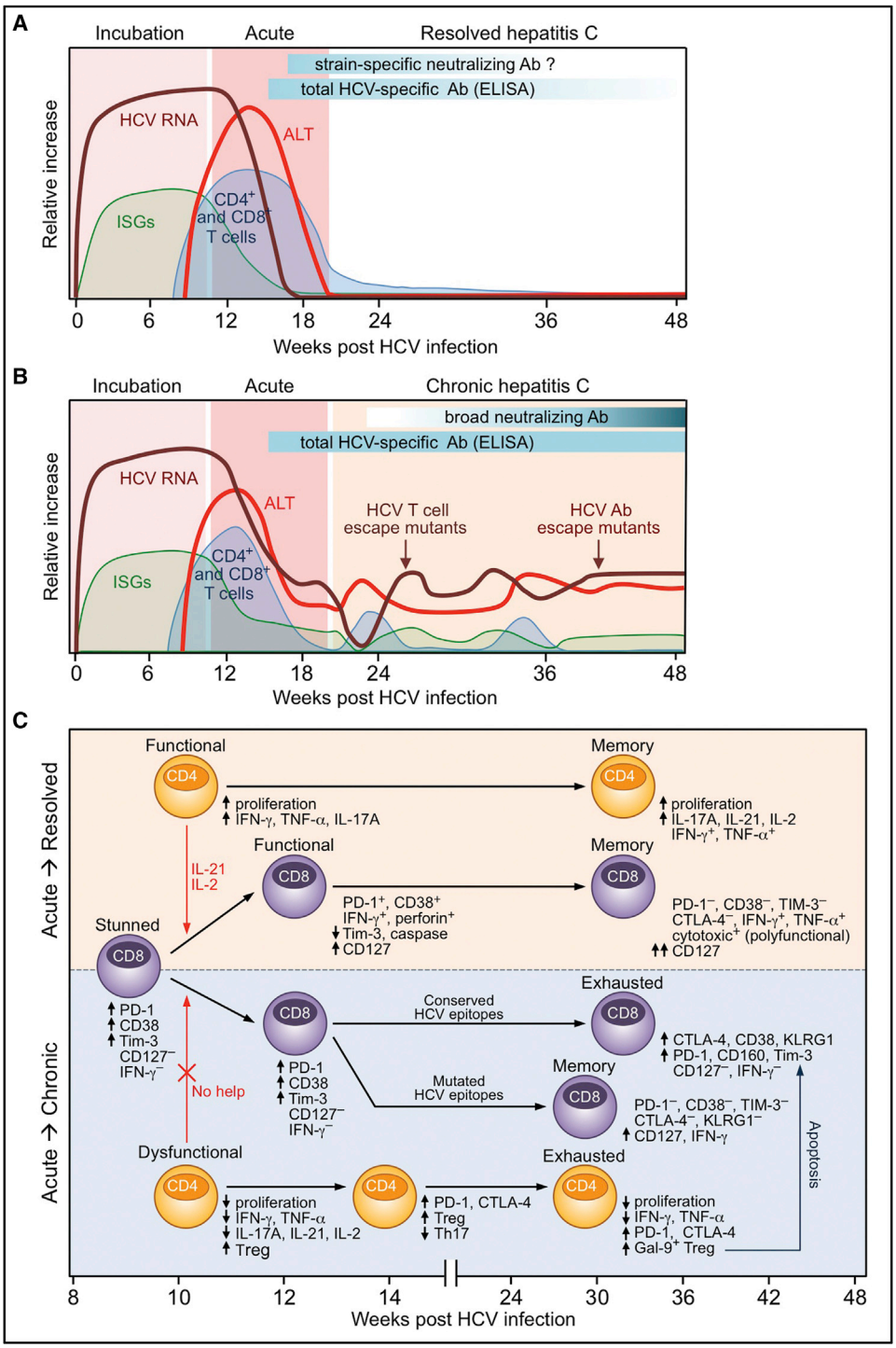

Figure 2. Clinical, Virological, and Immunological Course of Acute HCV Infection

(A) Acute hepatitis C followed by clinical recovery. The incubation phase with high viremia titer and ISG expression occurs during the first 8 weeks of infection. The acute phase is marked by elevated alanine aminotransferase (ALT) levels and the onset of HCV-specific $CD4^+$ and $CD8^+$ T cell responses around weeks 8 to 12. HCV-specific antibodies are detectable by enzyme immunoassay (ELISA) a few weeks later, but the impact of low titers of strain-specific neutralizing antibodies is still controversial. After spontaneous resolution of acute HCV infection, HCV-specific memory T cell responses remained detectable for decades whereas antibodies disappear from the circulation.

(B) Acute hepatitis C followed by chronic infection. HCV RNA titers decrease 2–3 $\log_{10}$ after the acute phase of hepatitis C and remain relative stable in the chronic phase. Selection of HCV escape mutants is mostly driven by T cells in the acute and early chronic phases of infection and by increasing titers of broadly targeted neutralizing antibodies in the chronic phase. The intensity of the shaded background indicates the degree of intrahepatic inflammation in the different phases of hepatitis.

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T cells are thought to contribute to HCV clearance because the increase in CD8β and IFN-γ mRNA expression in the liver coincides with a 90%–99% decrease in viral titer in acutely infected chimpanzees (Shin et al., 2006), and because depletion of either $CD4^+$ (Grakoui et al., 2003) or $CD8^+$ (Shoukry et al., 2003) T cells prevents HCV clearance in this model.

In patients with chronically evolving hepatitis, antigen-driven exhaustion and loss of IL-21-producing Th17 cells are thought to contribute to the failure to generate successful HCV-specific $CD8^+$ T cell responses (Kared et al., 2013). In vitro blockade of Tim-3, PD-1, and CTLA-4 increases the frequency of IL-17A- and IL-21-producing HCV-specific $CD4^+$ T cells in cultures of peripheral blood mononuclear cells that are obtained during the acute phase of infection (Kared et al., 2013), and IL-21 is sufficient to restore the in vitro proliferation of HCV-specific $CD8^+$ T cells (Kared et al., 2013; Radziewicz et al., 2007). In contrast, HCV-specific $CD8^+$ T cells coexpress PD-1, Tim-3, CD160, and 2B4 (CD244) in chronic infection (Figure 2C; Bengsch et al., 2010; Golden-Mason et al., 2007, 2009; Kroy et al., 2013; Nakamoto et al., 2009; Radziewicz et al., 2007; Schlaphoff et al., 2011) and combined PD-1 and CTLA-4 blockade is necessary to rescue their in vitro function (Nakamoto et al., 2009). When PD-1 signaling was blocked in vivo in three chimpanzees with chronic HCV, a significant but transient reduction in HCV viremia was observed in the animal that had the strongest and broadest $CD4^+$ and $CD8^+$ T cell response prior to development of chronic infection (Fuller et al., 2013). It is therefore conceivable that inhibitory molecules such as Tim-3 (McMahan et al., 2010) and additional factors, such as increased numbers of Treg cells and inhibitory cytokines (e.g., IL-10 and TGF-β) also contribute to the impaired T cell responses in chronic HCV infection (Franceschini et al., 2009; Golden-Mason et al., 2007; Radziewicz et al., 2007; Timm et al., 2004).

Although downregulation of $CD4^+$ and $CD8^+$ T cell responses against persistent antigens can be regarded as a host mechanism to minimize liver disease, HCV also exhibits specific strategies to escape from emerging cellular and humoral immune responses. This is facilitated by its high replication rate and the lack of proofreading by its polymerase. The selection of nonsynonymous mutations is highest in the acute phase of infection, and it is mostly mediated by $CD8^+$ T cells (Fuller et al., 2010). A mutation in a single T cell epitope often triggers the selection of several compensatory mutations to allow the virus to maintain its replicative fitness (Dazert et al., 2009). The effect of T cell selection pressure is illustrated by pregnancy-induced immune tolerance. The temporary decrease in adaptive immune responses results in a temporary reversion of HCV escape mutations and in an increase in HCV titer that lasts until T cell selection pressure is reimposed after childbirth (Honegger et al., 2013). A similar reversion of T cell escape mutants is also observed upon transmission of HCV into individuals that do not share the restricting HLA alleles with the source (Timm et al., 2004). However, in the absence of such changes in the infected host, HCV sequence evolution is predominantly driven by the antibody responses in the chronic phase of infection (Figure 2B) as a result of an increase in antibody breadth and titer (von Hahn et al., 2007).

Comparative Aspects of Innate and Adaptive Immune Responses in HAV, HBV, and HCV Infection

Because the acute phase of hepatitis C is clinically asymptomatic in most patients and HCV does not infect rodents, chimpanzees have been an important model to study the immune response in the first few weeks after infection. The same model has also been used in HAV and HBV infection to study intrahepatic immune responses early after infection.

Microarray analyses of prospectively collected chimpanzee liver biopsies demonstrate an induction of ISGs within days of HCV infection (Bigger et al., 2001; Su et al., 2002) along with a plateau but not decrease in viral titer (Figures 2A and 2B). Despite the early activation of innate interferon responses, it takes 8–12 weeks until adaptive immune responses appear. In most cases, these delayed HCV-specific T cell responses are weak and narrowly focused and do not clear the infection (Shin et al., 2013; Thimme et al., 2002). Likewise, neutralizing antibodies are rarely detectable and isolate-specific in chimpanzees (Logvinoff et al., 2004) and patients (Osburn et al., 2010; Pestka et al., 2007). As described in the previous section of this review, the primary defect may be in the HCV-specific $CD4^+$ T cell response.

Similar to HCV, HAV activates innate pattern recognition receptors in infected cells and cleaves downstream signaling molecules. The HAV dsRNA that is produced during its replication cycle is sensed by TLR3, and a short peptide at the 5′ end of the HAV RNA is sensed by the cytoplasmic RIG-I-like receptor melanoma differentiation associated gene 5 (Qu and Lemon, 2010). HAV also cleaves the adaptor molecules TRIF (Qu et al., 2011) and MAVS (Yang et al., 2007) downstream of these sensors. However, HAV is more successful in preventing innate immune responses than HCV, considering that HAV induces only a limited type I IFN response even with 100-fold higher intrahepatic vital titers (Lanford et al., 2011). It therefore comes as a surprise that HAV infection is self-limited rather than chronic. Viral clearance coincides with a strong HAV-specific response by IFN-γ-, IL-2-, and IL-21-producing $CD4^+$ T cells that appears 4–8 weeks earlier than in HCV infection (Zhou et al., 2012) and, along with a strong neutralizing antibody response, is associated with a decline in HAV viremia and lifelong protective immunity.

Acute HBV infection is also cleared by a strong adaptive immune response that confers lifelong protective immunity. Again, this is inversely correlated to the innate response during the first months of infection. HBV behaves as a stealth virus because it does not induce an ISG response when it spreads through the liver and infects almost 100% of the hepatocytes (Wieland et al., 2004). Its invisibility to the innate immune response has been attributed to its replication strategy. Replication occurs within viral capsids in the cytoplasm, and the generated viral RNAs are capped and polyadenylated, thereby resembling host mRNAs (Wieland et al., 2004). However, the absence of

(C) Adaptive immune responses associated with differential outcome of acute HCV infection. A strong and maintained $CD4^+$ T cell response appears to be a critical factor for the outcome of acute HCV infection. In its presence, HCV-specific $CD8^+$ T cell populations with an initially "stunned" phenotype acquire multiple effector functions (top). In the absence or loss of a strong $CD4^+$ T cell response, $CD8^+$ T cells develop exhausted phenotypes, which are attributed to chronic antigen-specific stimulation. Those $CD8^+$ T cells that target HCV escape variants remain functional with a memory phenotype in the chronic phase of infection (see text for details). Treg indicates regulatory T cell.

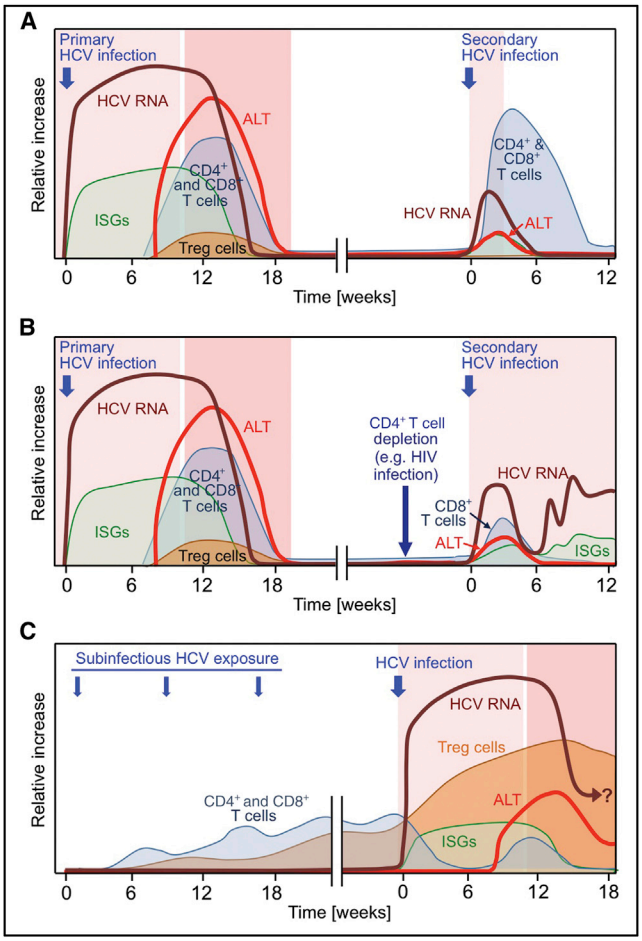

Figure 3. Conditions for T-Cell-Mediated Immune Protection upon HCV Reinfection

(A) Clearance of a primary HCV infection is associated with strong T cell responses. A secondary HCV infection results in lower HCV titers and more rapid viral clearance. An early memory T cell recall response is detectable in both liver and blood.

(B) Depletion of $CD4^+$ T cells in chimpanzees prior to secondary HCV infection results in weak recall responses, incomplete T-cell-mediated control of viremia, and emergence of MHC class I escape mutations. Chronic infection ensues.

(C) T cell responses that are induced by repeated exposure to trace amounts of HCV, in the absence of quantifiable systemic viremia and seroconversion ("subinfectious HCV exposure"), do not confer immune protection upon HCV reinfection. The absence of immune protection is associated with an expansion of Treg cells during subinfectious HCV exposure and subsequent HCV challenge. The intensity of the shaded background indicates the degree of intrahepatic inflammation in the different phases of hepatitis.

innate responses does not impair the induction of a vigorous HBV-specific $CD4^+$ T cell response, which appears much earlier than in HCV infection (Asabe et al., 2009). This $CD4^+$ T cell response determines the outcome of HBV infection because $CD4^+$ T cell depletion in the chimpanzee model abrogates the antiviral $CD8^+$ T cell response and results in chronic infection (Asabe et al., 2009).

Collectively, these observations raise the interesting hypothesis that the absence rather than presence of a strong type I IFN response is critical for the induction of a successful $CD4^+$ T cell response. This is consistent with data in LCMV-infected mice in which blockade of type I IFN signaling increases the frequency and function of virus-specific $CD4^+$ T cells (Teijaro et al., 2013; Wilson et al., 2013).

Conditions for Induction and Maintenance of Protective Immunity

Most vaccines, including those against HAV and HBV, induce an antibody response that is also observed in subjects with natural immunity. However, in contrast to the strong humoral immune responses in acute self-limited HAV and HBV infection, broadly neutralizing antibodies against HCV are generated only after months to years of chronic infection (Logvinoff et al., 2004). The recent resolution of the HCV E2 envelope core structure (Kong et al., 2013) may help to identify conserved structural determinants that are recognized by such broadly neutralizing antibodies and facilitate the design of immunogens to induce them. Additionally, an ideal HCV vaccine would induce T cell responses that are similar to those of patients who recover spontaneously from the infection. What is the evidence that such T cell responses are protective upon reinfection?

A small population of HCV-specific memory T cells persists for decades after spontaneous HCV clearance and can readily be expanded from the blood by in vitro stimulation with HCV antigens (Takaki et al., 2000). These cells protect HCV-recovered chimpanzees from developing chronic infection upon heterologous and cross-genotype HCV rechallenge as evidenced by lower amounts of viremia and faster clearance than in a primary infection and lack of liver disease (Figure 3A; Lanford et al., 2004; Nascimbeni et al., 2003). Depletion of either $CD4^+$ or $CD8^+$ T cells prior to rechallenge of these chimpanzees abrogates this protective but not sterilizing immunity (Figure 3B; Grakoui et al., 2003; Shoukry et al., 2003). In contrast to such controlled studies on primary and secondary infections in chimpanzees, prospective studies in humans underestimate the rate of T cell-based protection because they miss episodes of reinfection and clearance resulting from the short duration of viremia and the lack of clinical symptoms. However, it has been reported that clearance of a primary infection results in a greatly reduced chance of developing chronic hepatitis C in subsequent infections (Mehta et al., 2002; Osburn et al., 2010). This protection is lost upon infection with HIV and reduction of $CD4^+$ T cell counts, thus supporting a role of $CD4^+$ T cells (Mehta et al., 2002).

The conditions that result in such protective T cell responses need to be further defined. HCV-specific T cell responses are detected in some subjects without history of acute HCV infection despite frequent exposure (Mizukoshi et al., 2008; Thurairajah et al., 2008). Such T cells can indeed be induced in the absence of systemic viremia and seroconversion by repeated exposure to trace amounts of HCV as demonstrated in health care workers after accidental needlestick injury (Heller et al., 2013) and in the chimpanzee model (Park et al., 2013). However, such T cell responses do not protect chimpanzees upon subsequent challenge with HCV. Rather, HCV-specific recall and de novo responses, as well as intrahepatic T cell recruitment and IFN-γ production, are suppressed. This is associated with an increase in Treg cell frequency during subinfectious HCV exposure and subsequent HCV challenge (Figure 3C; Park et al., 2013). Thus, repeated low-dose exposure, as observed in endemic areas or in injection drug users, may not confer protective immunity but

instead expand Treg cells and suppress immune responses during subsequent acute infection. In addition, it should be noted that even proven protective immunity can be lost, e.g., by multiple sequential high-dose heterologous HCV challenges of a spontaneously recovered chimpanzee (Bukh et al., 2008).

Our current understanding that strong T cell-mediated immunity is crucial for protection against chronic HCV infection has informed vaccine studies. Vaccination with an adenovirus prime, DNA boost regimen induces strong HCV-specific T cell responses against HCV nonstructural antigens in chimpanzees, which develop a significantly shorter duration of viremia and lower HCV titer than do mock-vaccinated control chimpanzees upon subsequent HCV challenge (Folgori et al., 2006). Furthermore, alanine amino transferase (ALT) levels do not increase. Key to this protective immunity is the induction and early expansion of HCV-specific T cells with a high degree of functionality and high expression of the memory precursor marker CD127 (Park et al., 2012b). This is reminiscent of the early expansion of $CD127^+$ HCV-specific T cells with high functionality in chimpanzees that proceed to clear acute HCV infection (Shin et al., 2013). A similar approach induces multispecific and multifunctional HCV-specific $CD4^+$ and $CD8^+$ T cell responses in healthy subjects for at least a year after vaccination (Barnes et al., 2012).

Additional cues for the maintenance of strong immune responses may come from HBV infection. In contrast to HCV and HAV, HBV is not necessarily cleared completely in subjects who recover from acute infection. Rather, trace amounts of HBV DNA appear sporadically in the circulation (Rehermann et al., 1996) and cccDNA can persist in the form of a minichromosome (Levrero et al., 2009). These trace amounts of sporadically reappearing virus sustain HBV-specific humoral and cellular immune responses, and vice versa are controlled by them (Hoofnagle, 2009). This scenario is mimicked by the use of persistent CMV-derived vectors to deliver vaccine antigens. Although not yet tested in an HCV model, such vectors established indefinitely persistent, high-frequency, effector memory T cells in macaques vaccinated against simian immunodeficiency virus (SIV) and protect the animals upon a highly pathogenic SIV challenge (Hansen et al., 2011, 2013).

Concluding Remarks and Areas for Future Research

In the past 60 years, great progress has been made in understanding and treating viral hepatitis. This includes the molecular cloning of the parenterally transmitted HCV, HBV, and HDV and the enterally transmitted HAV and HEV, the development of protective vaccines against HAV, HBV, and HEV, the use of IFN-based therapies for all chronic hepatitis virus infections, and the design of antiviral agents that suppress HBV replication and clear HCV. For HCV, one of the most recently discovered hepatitis viruses, a detailed picture of its interaction with host innate and adaptive immune responses has emerged. These include mechanisms of inducing and evading strong innate interferon-based responses. Chronic innate immune activation, together with host genetic factors, negatively predict HCV clearance in response to IFN-based therapy. Although HCV persists in most infected individuals, successful adaptive immune responses were defined that not only clear a primary infection but also protect upon a secondary infection. The area that requires the greatest immunological effort is the development of an HCV vaccine. Apart from developing methods to induce protective memory T cell responses, this should include the characterization and induction of broadly neutralizing antibody responses. For HAV and HEV, a better understanding of the factors that drive the severe immunopathogenesis of fulminant hepatitis is necessary. Likewise, the immunopathogenesis of HDV infection, a virus that causes severe liver disease upon co- and superinfection with HBV, has not been characterized. With regards to HBV, a better understanding is needed of the factors that drive the onset of immuno-active disease in those individuals who are infected early in life. Because antivirals suppress but do not clear HBV infection, much remains to be done for those individuals who are already chronically infected and at risk of developing liver cirrhosis and hepatocellular carcinoma.

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