Failure of innate and adaptive immune responses in controlling hepatitis C virus infection

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Abstract
Effective innate and adaptive immune responses are essential for the control of hepatitis C virus (HCV) infection. Indeed, elimination of HCV during acute infection correlates with an early induction of innate and a delayed induction of adaptive immune responses. However, in the majority of acutely HCV-infected individuals, these responses are insufficient to clear the virus and persistence develops. In recent years, different mechanisms responsible for the failure of innate and adaptive immune responses have been identified. These include the proteolytic cleavage of molecules playing key roles in the induction of the interferon response, manipulation of interferon-induced effector proteins, interference with CD8+ T-cell function or immune escape in T- and B-cell epitopes. In this review, we discuss the possible roles of innate and adaptive immune responses in HCV clearance and the different evasion strategies used by the virus to escape these immune responses.

Introduction
Infections with the hepatitis C virus (HCV) are a leading cause of acute and chronic liver diseases. Acute hepatitis C is often asymptomatic or associated with only non-specific and mild symptoms; however, up to 80% of infections persist and these patients are at high risk to acquire serious liver damage including steatosis, liver cirrhosis, and hepatocellular carcinoma (reviewed in Lauer & Walker, 2001). Although HCV infection is an important predisposing condition to these manifestations, the likelihood for their development very much depends on cofactors including alcohol consumption, age, sex, and genetic predispositions such as distinct polymorphisms in genes involved in antiviral responses. The latter also plays a major role in determining the outcome of infection (acute self-limiting or chronic persistent), which ultimately results from a complex interaction between the virus and the host immune system.

It is estimated that c. 130 million people are persistently HCV-infected worldwide (reviewed in Shepard et al., 2005) and limited therapy options as well as the lack of a preventive vaccine aggravate this medical issue (reviewed in Lauer & Walker, 2001). Given this high frequency of persistence, HCV appears to possess efficient strategies to overcome immune responses. Thus, HCV is not only a medically highly relevant pathogen, but also an ideal model to study induction of and evasion from innate and adaptive immune reactions. In this review, we will summarize the current understanding of how HCV is able to escape from immune responses and to establish viral persistence.

Basic facts about the virus
HCV is an enveloped RNA virus that belongs to the Hepacivirus genus within the Flaviviridae family (Fig. 1a). The positive-strand genome has a length of c. 9.6 kb, and it encodes a polyprotein that is cleaved by viral and cellular proteases into 10 different proteins (reviewed in Pönnisch & Bartenschlager, 2010) (Fig. 1b). The structural proteins core, envelope protein 1 (E1) and E2 reside in the amino-terminal region of the polyprotein and they are the main constituents of infectious virus particles.
The hydrophobic p7 protein as well as nonstructural protein 2 (NS2) are required for virion assembly, but most likely are not part of the secreted particles. The serine-type protease residing in the amino-terminal NS3 domain forms a stable complex with the NS4A cofactor and catalyzes polyprotein cleavage at the NS3-4A, NS4A-B, NS4B-5A, and NS5A-5B sites. NS4B induces alterations of intracellular membranes, designated the membranous web, which are thought to be the site of viral RNA replication. The polyprotein is co- and post-translationally cleaved into 10 mature viral proteins: core (C), envelope protein 1 and 2 (E1 and E2), the ion channel protein p7 and non-structural proteins (NS) NS2, NS3, NS4A, NS4B, NS5A, and NS5B. Core, E1, and E2 build up the virus particle and are therefore called structural proteins, whereas the nonstructural proteins are not part of the virions but required for viral RNA replication. Functions ascribed to individual proteins are specified in the bottom.

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The primary target of HCV is the hepatocyte. Yet, several reports described the detection of viral RNA in peripheral blood mononuclear cells (reviewed in Zignego et al., 2007); however, in a comprehensive in vitro study, blood cells were demonstrated to be nonsusceptible to HCV infection and RNA replication (Marukian et al., 2008). This is also supported by the finding that scavenger receptor class B type 1 and claudin-1, two cell surface molecules participating in HCV entry, are highly expressed in liver cells (reviewed in Zeisel et al., 2011). Moreover, claudin-1 and occludin, yet another HCV entry molecule, participate in the formation of tight junctions, which are a well-established feature of hepatocytes, but cannot form in solitary cells such as blood cells. Nevertheless, in vitro replication of HCV in non-liver cells such as neuroepithelial cells has been described arguing that HCV is not strictly hepatotropic (Fletcher et al., 2010). Another hallmark of HCV is its narrow host range that is, at least in part, mediated by species determinants residing in the entry molecules CD81 and occludin (Ploss et al., 2009). Robust infections have only been described for chimpanzees, whereas other non-human primate species are non-permissive (Bukh et al., 2001) and infections of the non-primate mammal Tupaia belangeri are of limited efficiency and difficult to reproduce (Xie et al., 1998; Xu et al., 2007).
Overall, HCV replication is not cytolytic and persistent infection can be easily established in cultured cells. It is very likely that the same applies to infection in vivo arguing that liver cell destruction is caused primarily by host immune responses targeting HCV-infected cells rather than by the virus itself (reviewed in Chisari, 2005).

**Innate immune responses**

For many viruses, it has been shown that innate immune responses, most notably induction of type I and III interferons (IFNs), are the first line of defense limiting viral replication and spread, thus contributing to the outcome of an infection (reviewed in Haller & Weber, 2007; Randall & Goodbourn, 2008). The importance of IFNs is best illustrated by type I IFN receptor knockout mice, which quickly succumb to otherwise harmless viral infections although they have a fully functional adaptive immune system. Likewise, humans with genetic defects in the IFN system frequently die of viral diseases at an early age (Dupeux et al., 2003). Given the importance of this defense, viruses have evolved numerous counteracting strategies including the block of IFN induction, interference with signaling triggered by IFNs or inhibition of the action of one or several IFN-stimulated genes (ISGs) (reviewed in Haller & Weber, 2007; Randall & Goodbourn, 2008). As discussed below, HCV appears to be no exception to this rule.

**The IFN system: induction of antiviral cytokines**

IFNs that are expressed in a large variety of tissues are the type I IFNs, of which IFN-β is directly induced upon pathogen recognition, whereas IFN-α expression is primarily triggered by IFN-β. Very similar to IFN-β, IFN-λ, also designated type III IFN, is induced directly upon viral infection (Osterlund et al., 2007); however, in contrast to the virtually universally active type I IFNs, IFN-λ appears to have a more restricted tissue specificity (Sommerneys et al., 2008).

Type I IFNs comprise at least 13 IFN-α subtypes and one single IFN-β type, as well as some additional family members that are less involved in antiviral responses. Induction of type I IFN gene expression by the ‘classical pathway’ is best understood for IFN-β in fibroblasts. In infected cells, sensor molecules detect double-stranded RNA (dsRNA), which is generated as intermediates of viral transcription or replication (Fig. 2). Two intracellular RNA helicases of the retinoic acid inducible gene-I (RIG-I)-like receptor (RLR) family, RIG-I and Mda5 (melanoma differentiation associated gene 5), act as sentinels for viral dsRNA in the cytoplasm (reviewed in Yoneyama & Fujita, 2009). Then, activated RIG-I or Mda5 binds to the adapter mitochondrial antiviral signaling molecule (MAVS), formerly also known as IPS-1, Cardif or VISA. Via a rather complex assembly of different signaling mediators, including several members of the TNF-receptor associated factors (TRAFs), engagement of MAVS leads to the activation of two IκB kinase (IKK)-related kinases, IKKe and TANK-binding kinase-1 (TBK-1) (reviewed in West et al., 2011). They phosphorylate the C-terminal region of IFN regulatory factor 3 (IRF3) leading to its dimerization and consequent retention in the nucleus. IRF3 plays a central role in the activation of the IFN-β promoter and of a small subset of ISGs. Signaling that is triggered by this first wave of IFN leads to upregulation of the closely related transcription factor IRF7. It is activated in the same way as IRF3 and can homodimerize or heterodimerize with IRF3, leading to an amplification loop that initiates the synthesis of several IFN-α subtypes.

Interestingly, myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) express high levels of IRF7 even in the absence of microbial stimulation (reviewed in Lande & Gilliet, 2010). This enables them to directly produce high levels of IFN-α upon pathogen recognition, without the need of a first wave of IFN-β. Moreover, in addition to the cytoplasmic pathway of IFN induction, which requires intracellular replication of a virus, DCs sense viruses by extracytoplasmic Toll-like receptors (TLRs). They may serve as sensors for viral infection of phagcytosed cells; however, also intracellular virus replication can stimulate endosomal/lysosomal TLRs through autophagic engulfment of viral genomes and replication intermediates in the cytoplasm and delivery to TLR-containing autolysosomes (Lee et al., 2007). Human pDCs mostly express TLR7 and TLR9, which recognize single-stranded (ss) RNA and CpG DNA, respectively, whereas mDCs express TLR3, which responds to dsRNA. Signaling cascades triggered by activated TLRs differ in their use of adapter molecules from the cytoplasmic RLR pathway, involving factors such as MyD88 (TLR7 and 9) or TRIF (TLR3), but they eventually converge and lead to the activation of the same set of transcription factors comprising IRF3, IRF7, and NFκB (reviewed in Kawai & Akira, 2010).

**Interferon signaling**

IFN-α/β subtypes all bind to and activate a common type I IFN receptor consisting of two subunits (IFNAR-1 and IFNAR-2). IFN-α/β binding leads to heterodimerization of the IFNAR subunits and activation of the so-called JAK-STAT signaling pathway (reviewed in Schindler & Plumlee, 2008) (Fig. 2). The signal transducer and
activator of transcription (STAT) proteins are latent cytoplasmic transcription factors, which become phosphorylated by the Janus kinase (JAK) family members JAK-1 and TYK-2. Phosphorylated STAT-1 and STAT-2 recruit a third factor, IRF9 (also called ISGF3G or p48), to form a complex known as IFN-stimulated gene factor 3 (ISGF3). This heterotrimeric complex translocates into the nucleus and binds to IFN-stimulated response elements in the promoter regions of ISGs, thereby inducing their transcription.

Type III IFN comprises three subtypes of IFN-λ, namely IFN-λ1 (IL-29), IFN-λ2 (IL-28A), and IFN-λ3 (IL-28B) (Sheppard et al., 2003). All subtypes bind to a common heterodimeric receptor consisting of the IL-28 receptor alpha chain and the IL-10 receptor beta chain. Albeit engaging a distinct receptor, the signaling triggered by IFN-λ employs the same kinases and signal transducers of the JAK-STAT pathway as in type I IFN signaling. Moreover, the pattern of ISGs induced by IFN-λ in liver cells is very similar to the one triggered by IFN-α (Marcello et al., 2006).

To control and limit the positive feedback loop via IRF7, several specialized proteins serve as negative regulators of the JAK-STAT pathway. For example, the suppressor of cytokine signaling (SOCS) proteins are induced by STAT-signaling and specifically prevent further STAT activation by binding to activated cytokine receptors, inhibiting the activity of JAKs, and targeting bound signaling proteins for proteasomal degradation (Yoshimura et al., 2007). The constitutively expressed protein inhibitor of activated STAT (PIAS) family members function as small ubiquitin-like modifier (SUMO) E3 ligases and inhibit the transcriptional activity of STATs (Shuai & Liu, 2005).
Influence of the host IFN system on HCV clearance

Striking correlations between certain expression or sequence markers of the host IFN system and the outcome of HCV infection and therapy have been reported. Two studies have found that a marked upregulation of ISGs in the livers of HCV patients prior to therapy was associated with treatment failure (Chen et al., 2005; Sarasin-Filipowicz et al., 2008). In those patients, therapeutic administration of IFN-α could not increase the expression of ISGs above pre-treatment levels, which might indicate a certain degree of refractoriness of the IFN-system after prolonged preactivation (Sarasin-Filipowicz et al., 2008).

An even stronger association with viral clearance was found for a single-nucleotide polymorphism (SNP) in the promoter/enhancer region of the IL-28B gene (encoding IFN-λ3). Both the success rate of treatment (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009) as well as the rate of spontaneous virus clearance (Thomas et al., 2009) was shown to increase profoundly (greater than twofold) in patients with the C/C genotype. While the mechanism underlying this striking effect is still unclear, it highlights the role of the IFN system in the natural history of HCV infection.

Interferon effector proteins with antiviral activity against HCV

Both type I as well as type III IFNs are known to be effective against HCV in cell culture (Frese et al., 2001; Marcello et al., 2006; Diegelmann et al., 2010). IFN-α/β, and similarly IFN-λ, induce the expression of more than 300 genes, whose products have antiviral, antiproliferative, and immunomodulatory functions (reviewed in Randall & Goodbourn, 2008). IFN-induced proteins are a diverse class of factors of which only a few have been characterized in detail. In case of HCV, this is the case for protein kinase R (PKR) (Pflugheber et al., 2002), the dsRNA-specific adenosine deaminase 1 (ADAR1) (Taylor et al., 2005), the 2′−5′ oligoadenylate synthetases (OAS)/RNaseL system (Guo et al., 2004), the lipid droplet binding protein viperin (Helbig et al., 2005) and the IFN-induced protein with tetratricopeptide repeats 1 (IFIT1, ISG56, P56) (Wang et al., 2003).

PKR, ADAR1, and 2′−5′ OAS are constitutively expressed in normal cells, with their mRNA levels being upregulated by IFN-α/β. All three enzymes exist in a latent, inactive form, and need to be activated by viral dsRNA. PKR is a serine-threonine kinase that phosphorylates the alpha subunit of the eukaryotic translation initiation factor eIF2, thus slowing down RNA translation. ADAR1 catalyzes the deamination of adenosine on target dsRNAs to yield inosine, leading to the accumulation of mutations during viral replication. Upon dsRNA binding, 2′−5′ OAS catalyze the synthesis of short 2′−5′ oligoadenylates that activate the latent endoribonuclease RNaseL degrading both viral and cellular RNAs. In addition, degraded dsRNA might function as a stimulator of RIG-I, and could thereby represent a positive feedback mechanism for antiviral signaling (Malathi et al., 2007). IFT1 inhibits translation initiation at the level of eIF3 ternary complex formation and is likely to suppress viral RNA translation. Finally, viperin might block HCV replication by interfering with an important function of lipid droplets.

Further, a very recent study has approached antiviral activity of more than 300 ISGs against various viruses including HCV using a high-throughput, over-expression-based screening approach (Schoggins et al., 2011). Interestingly, beside a few virus-specific ISGs, that study predominantly identified broadly active key regulators of antiviral signaling as the most potent antiviral factors, such as RIG-I, Mda5, IRF1, IRF2, and IRF7 (IRF3 was not included, as it is not an ISG), which upon overexpression again trigger transcription of numerous target genes. This strongly corroborated the notion that there is not a single antiviral factor responsible for IFN-mediated inhibition of viral replication.

Apart from protein coding genes, IFN-induced regulation of miRNAs has been reported (reviewed in David, 2010). Some of these miRNAs appear to bind to and block the HCV RNA genome, whereas expression of the proviral miR-122 (Jopling et al., 2005) is reduced, arguing for a dual mode of antiviral action of IFNs at the level of miRNAs. While these are interesting observations, others did not find such a correlation (Sarasin-Filipowicz et al., 2009). Thus, further studies will be required to determine whether and to what extent miRNAs contribute to the IFN-induced block of HCV replication.

As mentioned earlier, hepatic ISG expression also before the onset of therapy can be observed in patients and, in fact, is commonly seen in HCV-infected chimpanzees particularly during the early phase of infection (Bigger et al., 2001, 2004; Su et al., 2002; Thimme et al., 2002; Major et al., 2004). This indicates that infected hepatocytes do respond to type I IFN; however, the source of the IFN is still under debate and could be either the infected hepatocytes themselves or bystander cells, such as pDCs infiltrating the liver (Bigger et al., 2001). In fact, in cell-culture pDCs can detect infected Huh-7 cells, sensing viral infection presumably via a TLR7-dependent pathway, and in response produce type I IFNs (Takahashi et al., 2010). Nevertheless, irrespective of the source of the IFN, in vivo these responses in a majority of cases do not eliminate the virus, suggesting that type I IFNs are
insufficient to completely control viral replication in infected hepatocytes. The underlying reasons are not clear, but several mechanisms have been suggested to contribute to this phenomenon that will be discussed in the following.

**HCV evasion from innate immune responses**

Contradicting reports exist as to whether HCV interferes with establishing an IFN-induced antiviral state of the host cell. Various studies described a partial block of JAK/STAT signaling in different cell lines and even in transgenic mice expressing HCV proteins or in cells harboring a genomic HCV replicon (Heim et al., 1999; Blindenbacher et al., 2003; Bode et al., 2003; Luquin et al., 2007). Several mechanisms have been suggested; for instance, it was reported that the HCV core protein upregulates the expression of SOCS 3, thereby inhibiting tyrosine phosphorylation of STAT-1 by the JAKs (Bode et al., 2003). Other studies have not detected a decrease in STAT-1 phosphorylation (Heim et al., 1999; Blindenbacher et al., 2003; Duong et al., 2004), but instead described an upregulation of the protein phosphatase PP2Ac, which indirectly led to the hypophosphorylation of STAT-1, favoring its association with PIAS1 and thereby inhibiting ISG transcription (Duong et al., 2004). This induction of PP2Ac levels was also observed to some degree in liver biopsies of HCV patients, where it similarly led to impaired ISG expression upon IFN-α treatment (Duong et al., 2004). While these studies are interesting, the fact remains that in cell culture, including various cell lines as well as primary human hepatocytes, HCV replication is highly sensitive to treatment with type I, II, or III IFNs (Frese et al., 2001, 2002; Marcello et al., 2006). It is, therefore, not clear whether modulation of IFN-signaling by HCV occurs, and if so, whether it would suffice to explain the failure of IFN to clear HCV.

Much better established is HCV interference with the induction phase of the antiviral response. It has been shown that the viral NS3/4A protease proteolytically cleaves central adapter molecules in two IRF3 activating antiviral pathways: MAVS in the cytosolic RLR pathway (Meylan et al., 2005) and TRIF in the endosome-borne TLR3 pathway (Li et al., 2005). For this reason, cells infected with HCV are impaired in their production of type I IFNs (Foy et al., 2003). Importantly, the observation that in HCV-infected Huh-7 cells MAVS is cleaved by the viral protease has been confirmed in humans (Bellecave et al., 2010). Using Western blot analyses of cell lysates prepared from liver biopsies, a convincing correlation was found between the degree of MAVS cleavage and HCV infection. Moreover, the amount of uncleaved MAVS was clearly reduced as compared to liver biopsies from patients with non-HCV liver diseases (Bellecave et al., 2010). Whether TRIF is also cleaved in vivo has not been determined thus far, but amino acids flanking the cleavage site are unfavorable arguing for a possibly low cleavage efficiency (Li et al., 2005).

It is feasible that the bulk of hepatic IFN is produced by infiltrating lymphoid cells, which likely cannot be productively infected and therefore would not be inhibited by NS3/4A action. Still, prevention of IRF3 activation is essential for the virus to achieve robust replication as demonstrated by forced stimulation of the RLR pathway in Huh-7 cells, which led to a marked reduction of intracellular viral replication (Binder et al., 2007). In RIG-I competent Huh-7 cells, HCV does not significantly activate IRF3 or induce ISG expression, at least at very early time points when MAVS could not plausibly be degraded by NS3/4A (Cheng et al., 2006; Binder et al., 2007). This finding indicates that HCV RNA genomes are rather weak RIG-I inducers, which might give the viral protease a head-start to inactivate the RLR-pathways before stimulatory dsRNA is generated as a result of RNA replication (Binder et al., 2007). We note that c. 1000 polyprotein molecules are translated per HCV genome, resulting in a rapid accumulation of NS3/4A even before the onset of active RNA replication (Quinkert et al., 2005). In fact, recent reports on RIG-I substrate recognition support this scenario, as it could be shown that RNA needs to present a 5'-terminal triphosphate group in the context of a short, blunt-ended double-strand, which is not present in the HCV genome (Brown et al., 1992; Schlee et al., 2009; Schmidt et al., 2009). Genome length dsRNA, which can be liberated during active RNA replication, however, has proven to be a prime trigger for RIG-I mediated IRF3 activation (Binder et al., 2011). It has been reported that the poly(U/UC) tract present in the 3' NTR of the HCV genome is a potent inducer of the RIG-I pathway (Saito et al., 2008; Uzri & Gehrke, 2009). However, in these studies, non-purified in vitro transcripts have been used. In the light of the results described earlier (Schlee et al., 2009; Schmidt et al., 2009) showing that dsRNA is frequently present as a side-product in unpurified in vitro transcripts, thus masking biological activity of ssRNA, the relevance of the poly(U/UC) tract for RIG-I activation needs to be revisited.

Attenuation of very early ISG expression by HCV might also be achieved through blocking RNA translation via PKR (Garaigorta & Chisari, 2009). It was found that HCV-infected Huh-7 cells treated with type I IFNs contain reduced levels of MxA and ISG15, although no reduction in transcription or nucleo-cytoplasmic transport of the corresponding mRNAs was observed. This reduction in ISG protein levels that is only seen in ‘acute’
infection, but not in a persistent state as mimicked with subgenomic replicons, was linked to phosphorylation (activation) of PKR, resulting in phosphorylation (inhibition) of eIF2α. Non-phosphorylated eIF2α is essential for translation of capped mRNA, including the ones of the ISGs, whereas certain non-capped RNAs such as the HCV genome are translated independently from this factor. In this model, HCV would block translation of ISG mRNAs without affecting synthesis of its own polyprotein. Another study describes similar findings, but PKR activation was very transient and translational inhibition was only observed between 9 and 18 h after infection (Arnaud et al., 2010). Moreover, contradictory reports have been published as to the role of PKR in controlling viral replication. For instance, an inhibition rather than the enhancement of HCV replication by phosphorylated PKR has been described (Kang et al., 2009). In addition, several groups reported that HCV resistance to type I IFNs is mediated through suppression of PKR kinase activity (Gale & Katze, 1998; Taylor et al., 1999). Apart from technical reasons, such as the use of different cell lines or different HCV expression/repllication systems, the time point when type I IFNs are added might be crucial. Indeed, early addition may block HCV replication potently and reduce viral RNA to a minimum level inducing only weak phosphorylation of PKR and accordingly little suppression of other ISGs. In contrast, if type I IFN is added at the peak of viral replication, HCV RNA levels cannot be decreased as potently, leading to substantial induction of phospho-PKR and, thereby, maximal suppression of ISG expression. Moreover, beside phosphorylated eIF2α, activated PKR assists in the production of autocrine IFN, thus accelerating the establishment of an antiviral state (Balachandran et al., 2000). The balance between these various functions of PKR may determine its pro- or antiviral activity.

In addition to the aforesaid classical pathways of innate antiviral immunity, autophagy, an essential catabolic process of eukaryotic cells, is increasingly recognized as a key player in this defense (reviewed in Saitoh & Akira, 2010). Many viruses, including HCV, were reported to depend on, but at the same time be controlled by autophagic processes (reviewed in Sir & Ou, 2010). It is known that HCV replication triggers the unfolded protein response (Tardif et al., 2002), which in turn leads to the induction of autophagy (Ait-Goughoulte et al., 2008; Sir et al., 2008). While this is generally looked at as another intrinsic antimicrobial defense strategy of the host, HCV might exploit this response for some early steps in its replication cycle (Dreux et al., 2009). Interestingly, two other groups could show recently that induction of autophagy by HCV might be directly involved in the suppression of type I IFN production, as RIG-I stimulation in cells with a knock-down of key regulators of autophagy yielded significantly higher induction rates of IFN-β (Ke & Chen, 2011; Shrivastava et al., 2011). Taken together, HCV appears to use several strategies to reduce immuno-recognition, thereby preventing rapid production of antiviral cytokines (Fig. 2). This might allow the virus to install its robust, persistent replication machinery, before the host detects its presence. While this is likely true for single infected cells, the initial inconspicuousness of the infection, with its putatively very low cytokine profile, might also delay the activation of other crucial branches of the immune system, such as natural killer (NK) cells as well as cellular and humoral responses of the adaptive immunity that will be discussed in the following sections.

**Role of NK cells in HCV infection**

NK cells are reported to play an important role in the innate immune response to HCV infection (Cheent & Khakoo, 2011). Several activating as well as inhibitory receptors, including killer immunoglobulin-like receptors (KIR) are involved in their regulation. Various KIRs can interact with HLA class I molecules, leading to differential levels of NK cell inhibition or activation. For instance, KIR2DL3, an inhibitory receptor, binds to HLA class I molecules encoded by HLA-C group 1 alleles (HLA-C alleles with a serine at position 77), triggering a comparatively weak inhibitory signal. A combined homozygosity for these two alleles (KIR2DL3 and HLA-C group 1) has been described by Khakoo et al. to exhibit a strong association with viral clearance: 19.4% of patients with cleared HCV infection, but only 12.3% with chronic infection were homozygous for both loci (OR 1.71, p 0.003) (Khakoo et al., 2004). They speculated that the threshold for NK cell activation might be lower in these patients allowing for stronger and more rapid antiviral effector functions. Curiously, this association was not evident in blood transfusion-borne infections, arguing that the innate immune system in these patients was overwhelmed. It should be noted that this association was confirmed by some (Romero et al., 2008; Knapp et al., 2010) but not all studies (Montes-Cano et al., 2005; Rauch et al., 2007), which might be due to different ethnic backgrounds of the cohorts and/or limitations in cohort size.

A possible role of NK cells in HCV immunobiology is further supported by the finding that they are activated in acutely infected subjects, as determined by an increased expression of the activating receptor NKG2D on both CD56bright and CD56dim subsets of NK cells. This is accompanied by an increased production of IFN-γ and cytotoxicity (Amadei et al., 2010). Although the overall killing capacity of NK cells was not impaired or even
enhanced in chronically infected patients (Golden-Mason et al., 2008; Oliviero et al., 2009; Ahlenstiel et al., 2010; Amadei et al., 2010), it is not clear whether the ability of NK cells to produce antiviral cytokines during chronic infection is altered. Two groups reported impairment of IFN-γ production (Oliviero et al., 2009; Ahlenstiel et al., 2010), while two other groups came to the opposite conclusion (Golden-Mason et al., 2008; Amadei et al., 2010). Of note, cytokine-stimulated NK cell lines and primary NK cells isolated from healthy donors can lyse HCV-replicating cells, particularly at high effector-to-target ratios (Larkin et al., 2006; Stegmann et al., 2010) and also secrete IFN-γ that mediates the inhibition of HCV replication (Li et al., 2004).

So far, there are only few studies indicating that HCV has the ability to interfere with the action of NK cells. A recent report suggests that NS5A-containing apoptotic bodies can trigger monocytes to produce increased amounts of IL-10 and decreased levels of IL-12. In consequence, this leads to a significant down-regulation of NKG2D on NK cells via TGF-β (Sene et al., 2010). Another proposed mechanism for HCV-induced NK cell inhibition is crosslinking of CD81 by the HCV envelope protein E2. It has been shown that engagement of this tetraspanin on the surface of NK cells exerted an inhibitory effect, leading to decreased cytotoxicity and IFN-γ production (Crotta et al., 2002; Tseng & Klimpel, 2002). Nonetheless, this effect could only be observed at high concentrations of soluble E2 protein or HCV virions immobilized to the cell-culture plate, but not by direct exposure of NK cells to infectious HCV particles, leaving the question open, as to whether this phenomenon could play a physiological role in vivo (Yoon et al., 2009; Crotta et al., 2010; Farag et al., 2011).

Adaptive immune responses

It is generally accepted that adaptive immune responses play a central role in disease pathogenesis and outcome (clearance vs. persistence) in patients with HCV infection (Rehermann, 2009; Walker, 2010). Most likely, multiple components of the adaptive immune system are involved in viral control and finally clearance, including humoral antibody responses and T cells.

Humoral antibody responses

Most acutely HCV-infected individuals produce antibodies against epitopes within the structural as well as non-structural proteins. Of note, however, most of them have no relevant antiviral activity, and only a small fraction of antibodies is able to inhibit virus binding, entry, or uncoating. These antibodies can potentially block HCV infection and are called ‘neutralizing antibodies’. First evidence for the existence of neutralizing antibodies in HCV infection came from studies in experimentally HCV-infected chimpanzees (Farci et al., 1994), and the association of antibodies targeting similar epitopes with viral clearance in patients suggested that neutralizing antibodies also exist in men. Using different in vitro models, multiple linear, conformational, and discontinuous epitopes targeted by neutralizing antibodies have been identified in the envelope glycoproteins E1 and E2, with a ‘hot-spot’ in and adjacent to hypervariable region 1 (HVR-1) (Walker, 2010; Sabo et al., 2011). These epitopes may have important functions in virus binding and entry, and antibodies directed against them may inhibit infection also at a post-attachment step (Sabo et al., 2011).

The role of neutralizing antibodies in acute HCV infection, and most importantly, in viral clearance is not completely understood. Many studies have suggested that the majority of patients with acute-resolving HCV infection lack neutralizing antibodies, whereas patients with a chronic course of infection develop such antibodies after viral persistence has been established (Bartosch et al., 2003; Logvinoff et al., 2004; Netski et al., 2005; Kaplan et al., 2007; Zeisel et al., 2008). However, one study reported that during acute HCV infection, virus-specific neutralizing antibodies force sequence evolution in vivo and, in some individuals, may play a role in determining the outcome of infection (Dowd et al., 2009). It has also been shown that HCV reinfection and subsequent viral clearance is associated with the generation of cross-reactive humoral responses (Osburn et al., 2010). Nonetheless, results from the study of a well-characterized and homogenous group of young women infected with the same HCV inoculum (strain AD78) by a contaminated anti-D immunoglobulin preparation suggest the development of neutralizing antibodies in the early phase of infection in the majority of patients, who are able to clear the infection spontaneously (Pestka et al., 2007). In contrast, a delayed induction of neutralizing antibodies was observed in patients with a chronic course of infection (Pestka et al., 2007) arguing for an important role of an early neutralizing antibody response in HCV clearance. However, it remains unknown whether the neutralizing antibody response really mediates viral clearance. We note that resolution of HCV infection has also been observed in the absence of neutralizing antibodies and even in hypoglobulinaemic individuals (Walker, 2010).

HCV evasion from humoral antibody responses

In chronic infection, HCV-specific neutralizing antibodies can be detected in most patients. However, multiple
mechanisms for the failure of the humoral immune response have been suggested. For example, evolution of viral quasispecies within targeted epitopes may lead to escape from neutralizing antibodies (Farci et al., 1996). HVR-1 may also play a more general role in mutational escape by serving as a decoy for neutralizing antibodies thus ‘protecting’ other functionally important, but less mutable epitopes (von Hahn et al., 2007). Interactions of HCV glycoproteins with high-density lipoprotein (HDL) and the scavenger receptor B1 (SCARB1) participating in HCV entry may protect from neutralizing antibodies (Logvinoff et al., 2004). In addition, specific glycans on E2 also modulate cell entry and confer protection from neutralizing antibodies (Falkowska et al., 2007; Helle et al., 2007) and conformational changes or binding of non-neutralizing antibodies may prevent binding of neutralizing antibodies (Zhang et al., 2007). It has also been proposed that HVR-1 obstructs the viral CD81 binding site and conserved neutralizing epitopes (Bankwitz et al., 2010). Interestingly, recent studies indicate that HCV may also evade neutralization by direct cell-to-cell transfer of the virus (Timpe et al., 2008; Witteveldt et al., 2009; Brimacombe et al., 2011).

**T-cell responses**

Several studies have shown that HCV elimination is associated with strong and sustained CD4+ and CD8+ T-cell responses that target multiple epitopes within the different HCV proteins (Diepolder et al., 1995, 1997; Missale et al., 1996; Cooper et al., 1999; Lechner et al., 2000; Thimme et al., 2001, 2002; Day et al., 2002; Cox et al., 2005a). Of note, with a delay of approx. 6–8 weeks, T cell responses become detectable and coincide with the onset of hepatitis. Recent data suggests that this is indeed because of delayed induction and not an impaired recruitment of specific CD8+ T cells to the liver (Shin et al., 2011). Upon resolution of the infection, these responses persist for decades and can even outlive humoral responses (Takaki et al., 2000).

There is good evidence supporting an important role for both T-cell subsets in controlling HCV infection. As for CD8+ T cells, the following findings support this notion:

First, there is a clear kinetic correlation between the onset of virus-specific CD8+ T-cell responses and HCV clearance. As shown by several groups, vigorous peripheral and intrahepatic virus-specific CD8+ T-cell responses targeting multiple epitopes are temporally associated with the onset of liver disease and viral clearance (Cooper et al., 1999; Gruner et al., 2000; Lechner et al., 2000; Thimme et al., 2001, 2002; Cox et al., 2005a).

Second, a strong association is found between defined HLA class I alleles and spontaneous resolution of the infection. As CD8+ T cells recognize antigens in the context of their presenting HLA class I molecule, it had been suggested early on that certain alleles would be associated with the outcome of HCV infection, that is viral clearance or development of persistence. Importantly, in an Irish cohort of women accidentally infected with HCV genotype 1b from a single source more than 20 years ago, the role of HLA alleles for the outcome of HCV infection could be clearly demonstrated (McKiernan et al., 2004): HLA class I alleles A3, B27 and Cw*01 were associated with viral clearance, whereas B8 was associated with viral persistence. The strongest protective effect was observed for HLA-B27, and this effect could be linked to an HLA-B27-restricted HCV epitope that predominated in HLA-B27-positive Irish women who had cleared the infection (Neumann-Haefelin et al., 2006). Of note, the protective effect of HLA-B27 requires the presence of a genotype-specific immunodominant CD8+ T-cell epitope and is thus absent in genotype 3–infected patients (Neumann-Haefelin et al., 2010). A similar HLA-allele associated and CD8+ T-cell epitope-mediated effect could also be shown for HLA-B57 and HLA-A3 (Thio et al., 2002; Kuniholm et al., 2010; Fitzmaurice et al., 2011; Kim et al., 2011). These combined results clearly support the dominant role of virus-specific CD8+ T-cell responses in HCV infection.

Third, virus-specific CD8+ T cells mediate strong antiviral effector functions in vitro. This was shown by using a novel immunological model based on a subgenomic replicon-containing cell line that was stably transduced with the common MHC class I allele HLA-A2 gene. Using this model, we could show that HCV-specific CD8+ T cells exert strong antiviral effects primarily through IFN-γ-mediated non-cytolytic effector functions and only to a lower extent through cytolytic effector functions (Jo et al., 2009, 2011).

Fourth and most importantly, CD8+ T cells have been shown to control HCV replication in vivo. Upon antibody-mediated depletion of CD8+ T cells, experimental infection of a chimpanzee led to HCV persistence until CD8+ T-cell response recovered and an HCV-specific CD8+ T-cell response emerged (Shoukry et al., 2003). Collectively, these results show that virus-specific CD8+ T cells are most likely the key effector cells in HCV control.

While most studies have been focused on the analysis of IFN-γ producing CD8+ T cells, it is important to note that recent studies have also demonstrated the presence of HCV-specific IL-17 producing CD8+ T cells that are characterized by high expression of CD161 and chemokine receptors such as CXR6 that is important for liver homing (Northfield et al., 2008; Billerbeck et al., 2010). These cells might have a protective role in HCV infection,
but additional studies are required to confirm this interesting hypothesis. Interestingly, we could recently show that virus-specific IL-17 producing CD8+ T cells recognize different HCV specific antigens as compared to IFN-γ producing CD8+ T cells (Grafmüller et al., 2011). In addition to CD8+ T cells, HCV-specific CD4+ T cells appear to contribute to HCV clearance. Indeed, several studies in patients with acute HCV infection revealed that a strong, multi-specific and sustained HCV-specific CD4+ T-cell response is associated with a self-limited course of infection (Diepolder et al., 1995, 1997; Missale et al., 1996; Gerlach et al., 1999; Day et al., 2002; Schulze Zur Wiesch et al., 2005). In addition, similar to the findings described for HLA class I, certain HLA class II alleles have been correlated with the outcome of HCV infection. For example, in heterogenous study cohorts, the HLA class II alleles most reproducibly associated with viral clearance are DRB1*1101 and DQB1*0301, which are genetically closely linked, a phenomenon referred to as linkage disequilibrium (Hong et al., 2005). In the well-defined Irish cohort (see above), however, DRB1*0401, DRB1*0401, and DRB1*15 correlate with protection (McKiernan et al., 2004). It is intriguing that most of the CD4+ T-cell epitopes identified thus far are restricted by HLA alleles for which a protective effect has been shown. Nevertheless, we note that CD4+ T-cell epitopes are highly promiscuous and can often be restricted by multiple different HLA class II molecules (Walker, 2010). In strong support of the important role of CD4+ T cells, their depletion in previously protected chimpanzees led to HCV persistence and the emergence of CD8+ escape variants (Grakoui et al., 2003). Finally, as has been described for CD8+ T cells, IL-17 producing CD4+ T cells have also been found in chronic infection, but their role in immune control remains to be determined (Rowan et al., 2008). Collectively, these findings clearly indicate that virus-specific CD4+ T cells are central regulators, while virus-specific CD8+ T cells function as the key effectors.

Evasion from T-cell responses

Virus-specific T-cell responses are also detectable during chronic HCV infection where they probably contribute to the progression of liver disease (Lauer et al., 2002; Neumann-Haefelin et al., 2008). Some patients with chronic HCV infection, however, lack strong and multi-specific CD8+ T-cell responses. In these patients, it is difficult to distinguish whether virus-specific CD8+ T-cell responses were not primed initially (primary T-cell failure) or whether they were primed, but disappeared quickly (T cell exhaustion). Results obtained from the early phase of acute HCV infection in chimpanzees (Cooper et al., 1999; Thimme et al., 2002) and in healthcare workers infected via needlestick exposure (Thimme et al., 2001) support the hypothesis that at least in some patients CD8+ T cells

![Fig. 3. Strategies used by HCV to interfere with adaptive immune responses.](image-url)
are not or weakly primed during acute HCV infection. Impaired priming of HCV-specific CD8+ T cells might be mediated by low numbers or functional impairments of antigen-presenting cells such as macrophages or dendritic cells (Walker, 2010). However, this issue remains controversial.

In most chronically HCV-infected patients, virus-specific T cells are present and even enriched in the liver, but they are unable to clear the infection (Walker, 2010). The mechanisms responsible for the failure of HCV-specific CD4+ and CD8+ T-cell responses during chronic infection are not well defined, but two main reasons appear to be responsible that are discussed in detail in the next two sections (see also Fig. 3).

**Viral escape**

HCV is an RNA virus with a very high replication rate of an estimated 10^{12} virions (i.e. genome equivalents) per day and person (Neumann et al., 1998), mediated by an RNA-dependent RNA polymerase lacking proof-reading function. Therefore, multiple virus variants co-circulate in an individual patient facilitating the selection of CD8+ T-cell escape variants. Indeed, first evidence for viral escape in HCV infection came from studies of chronically infected patients (Chang et al., 1997) and experimentally infected chimpanzees (Weiner et al., 1995; Erickson et al., 2001). Importantly, viral escape mutations seem to emerge during the acute phase of infection and are associated with chronicity (Erickson et al., 2001). In patients with acute HCV infection, viral escape from CD8+ T-cell responses was primarily detectable in patients developing chronic hepatitis C, but not in individuals who resolved infection (Timm et al., 2004; Cox et al., 2005a, b; Tester et al., 2005). Interestingly, many mutations outside of targeted CD8+ T-cell epitopes corresponded to conversion to the consensus sequence, indicating that because of the lack of sufficient T-cell-mediated selective pressure, HCV reverts to the fittest sequence, most likely represented by the consensus (Cox et al., 2005b). This concept has been further supported by a study showing that the transmission of an HLA-B8-associated escape mutation to an HLA-B8 negative subject resulted in rapid reversion of the mutation (Timm et al., 2004). These results were supported by a study using the cohort of Irish women described earlier; amino acid substitutions in known epitopes were directed away from consensus present in the inoculum in women having the HLA allele associated with that epitope, but toward the consensus in those women lacking the allele (Ray et al., 2005). These findings are in agreement with the concept of viral fitness cost, indicating that viral escape mutations are often associated with a reduced replicative capacity of the virus (Bowen & Walker, 2005).

Based on the finding that immunodominant CD8+ T-cell epitopes leave their viral footprint sequences in HCV genomes replicating during the chronic phase, virus genome sequencing studies were performed in order to identify footprints of additional potential CD8+ T-cell epitopes (Gaudieri et al., 2006; Timm et al., 2007; Rauch et al., 2009; Ruhl et al., 2011). Indeed, these studies identified additional HLA allele-dependent polymorphisms and thus candidate CD8+ T-cell epitopes. Importantly, the strongest association between specific sequence variations and an HLA allele in the study by Timm and co-workers (Timm et al., 2007) was found in a viral region that was shown to contain an immunodominant HLA-B27-restricted CD8+ T-cell epitope (Neumann-Haefelin et al., 2006). These results support the concept that CD8+ T-cell mediated pressure and viral escape is operative in a large fraction of patients.

Different molecular mechanisms are operating by which a certain mutation results in escape from CD8+ T-cell response. For example, mutations located at the HLA binding anchors, usually P2 and the C-terminal amino acid, strongly attenuate peptide binding to the HLA molecule. In contrast, mutations residing in the centre of the epitope are more likely to interfere with T-cell receptor (TCR) recognition (Soderholm et al., 2006). Mutations in the flanking region may prevent posttranslational processing of the peptide precursor (Seifert et al., 2004; Timm et al., 2004; Kimura et al., 2005).

The factors promoting or limiting the emergence of viral escape are poorly understood. In the chimpanzee model, it has been shown that upon depletion of CD4+ T cells, in the acute phase of infection viral escape from the CD8+ T cell response occurs and is associated with chronicity (Grakoui et al., 2003). This finding has led to the hypothesis that viral escape within CD8+ T-cell epitopes may be a consequence of insufficient CD4+ help. Other studies indicate that a limited TCR diversity might be associated with viral escape (Meyer-Olson et al., 2004). Of note, viral escape does not seem to occur in the context of dysfunctional CD8+ T-cell responses (Urbani et al., 2005). The strong association between specific HLA alleles and viral escape within an immunodominant HLA-restricted epitope indicates that the restricting HLA allele background also plays an important role in determining viral escape (Schmidt et al., 2011).

As mentioned earlier, another important determinant of viral escape is fitness cost, limiting the ability of the virus to tolerate a given escape mutation. Indeed, fitness cost might not only explain the occurrence of reversion after removal of T-cell pressure or the absence of viral escape in specific CD8+ T-cell epitopes, but also directly contributes...
to the protective effect of specific CD8+ T-cell responses. This has been suggested to be the case for the protective HLA-B27 and HLA-A3 epitopes (Dazert et al., 2009; Fitzmaurice et al., 2011). For instance, viral escape mutations affecting the main HLA-B27 anchor residues at position 2 and 9 of the dominant HLA-B27 epitope impair HCV replication (Dazert et al., 2009). This epitope is located within the RNA-dependent RNA polymerase (NS5B) region and mutations affecting this epitope impair viral fitness. Nevertheless, nearly all HLA-B27+ patients with persistent HCV genotype 1 infection display clustered viral sequence variations consistent with HCV escape mutations in this otherwise highly conserved region that, however, spare the HLA binding anchor residues. This occurrence of clustered mutations is a striking finding, as viral escape mutations in HCV epitopes restricted by other HLA alleles usually only affect a single amino acid residue within the epitope (Dazert et al., 2009). Importantly, this clustering of mutations is required for efficient CD8+ T-cell escape because of broad cross-recognition of viral variants (Dazert et al., 2009). Taken together, these results clearly illustrate that escape from the immunodominant HLA-B27-restricted CD8+ T-cell response is determined by counteracting factors: fitness cost and broad cross-recognition requiring clustered mutations. Thus, immune escape probably takes too long in most HLA-B27+ patients, providing the host immune system enough time to clear the virus before escape can take place. Interestingly, a similar observation has been reported for HLA-A*03-restricted CD8+ T cells that has been described to be protective in the Irish cohort of women infected with HCV genotype 1b from a single source (McKiernan et al., 2004). Indeed, Merani and co-workers studied HLA-class I–driven viral sequence diversity in this cohort and the strongest association between any HLA class I allele and a sequence mutation was found within an HLA-A*03-restricted CD8+ T-cell epitope in NS3 (NS31086–1088; amino acid sequence TVYH- GAGTK) (Merani et al., 2011). Similar to escape from the immunodominant HLA-B*07 epitope, viral sequence mutations within the HLA-A*03 epitope were clustered at positions 1087 and 1088 in most A*03+ patients (Merani et al., 2011). A subsequent study by the group of Paul Klenerman revealed that HLA-A3 restricted CD8+ T-cell responses indeed target these key epitopes and that two mutations are required to retain viral fitness (Fitzmaurice et al., 2011). Thus, clustered escape mutations occurring in both, the protective HLA-B*27 and HLA-A*03 restricted epitopes, argue for a complex pathway of viral escape requiring clustered mutations that may indeed be a major determinant of protective HLA class I alleles in HCV infection.

It is important to note that viral escape is not limited to CD8+ T-cell epitopes. However, although escape muta-

tions can occur in MHC class II–restricted epitopes, they are rarely found in chronically infected patients and chimpanzees (Fleming et al., 2010; Fuller et al., 2010), suggesting that other mechanisms such as CD4+ T-cell dysfunction might be responsible.

Although in the past few years important insights into CD8+ T-cell-mediated viral escape has been gained, we still do not know whether viral escape is a cause or consequence of HCV persistence. We also note that viral escape is not a universal mechanism resulting from CD8+ T-cell pressure. Indeed, combined immunological and virological studies suggest that viral escape occurs in 50–70% of targeted CD8+ T-cell epitopes (Cox et al., 2005b; Neumann-Haefelin et al., 2008). Thus, other mechanisms such as CD8+ T-cell dysfunction contribute to CD8+ T-cell failure as well.

**CD8+ T-cell dysfunction**

Several groups have suggested that CD8+ T-cell dysfunction, for example the inability to secrete antiviral cytokines such as IFN-γ or to proliferate in response to antigen contact is a major determinant of viral persistence (Wedemeyer et al., 2002; Spangenberg et al., 2005; Penna et al., 2007; Radziewicz et al., 2007, 2008; Nakamoto et al., 2009). This state of T-cell dysfunction observed during chronic viral infections is characterized by an up-regulation of inhibitory receptors, such as PD-1. Indeed, in chronically HCV-infected patients, several groups have shown that a large fraction of HCV-specific CD8+ T cells are also characterized by a high expression of PD-1 (Golden-Mason et al., 2007; Penna et al., 2007; Radziewicz et al., 2007, 2008; Nakamoto et al., 2008). These HCV-specific CD8+ T cells also display a low expression of CD127 (Bengsch et al., 2007; Golden-Mason et al., 2007; Radziewicz et al., 2007). Intrahepatic HCV-specific CD8+ T cells with a high PD-1 expression are prone to apoptosis (Radziewicz et al., 2008). Importantly, the impaired proliferative response of CD127-PD-1+ HCV-specific CD8+ T cells to antigenic stimulation can be increased by blocking antibodies targeting PD-1 (Golden-Mason et al., 2007; Penna et al., 2007; Radziewicz et al., 2007). These findings strongly indicate that T-cell exhaustion occurs during chronic HCV infection and that blockade of inhibitory receptor pathways may represent a novel therapeutic strategy for the augmentation of T-cell responses during HCV infection. However, the dysfunction of CD127 cells is not solely caused by inhibitory signals via PD-1, as PD-1 blockade alone was unable to restore the function of strongly inhibited HCV-specific CD8+ T cells in the liver (Nakamoto et al., 2008). Of note, targeting the additional inhibitory receptor CTLA-4 resulted in an increase in T-cell function
(Nakamoto et al., 2009). These findings – together with the observation that PD-1 expression on HCV-specific CD8+ T cells did not necessarily identify exhausted T cells during acute HCV infection (Bowen et al., 2008; Kasprovicz et al., 2008) – suggested that pathways other than PD-1 play a role in the dysfunction of CD127– HCV-specific CD8+ T cells. For example, expression of the negative immune regulatory receptor Tim-3 has been detected with HCV-specific CD8+ T cells in chronic infection (Golden-Mason et al., 2009) and its blockade may restore HCV-specific CD8+ T dysfunction (McMahan et al., 2010; Callendret & Walker, 2011). Similarly, a recent study has suggested a role for 2B4 in HCV-specific CD8+ T-cell dysfunction (Schlaphoff et al., 2011). However, the relative contribution of different inhibitory receptors for HCV-specific CD8+ T-cell dysfunction is not clear. Recently, CD127low HCV-specific CD8+ T cells were shown to coexpress the inhibitory receptors 2B4, KLRG1, and CD160 in addition to PD-1 in chronic HCV infection (Bengsch et al., 2010). Importantly, such a coexpression of multiple inhibitory receptors in addition to PD-1 was also found with highly exhausted T cells during chronic LCMV infection. Indeed, in that model exhausted T cells required the simultaneous blockade of additional inhibitory pathways to restore antiviral function (Blackburn et al., 2009).

In agreement with this finding, CD127– HCV-specific CD8+ T cells coexpressing multiple inhibitory receptors could be partly, but not completely, reinvigorated by PD-1 blockade alone (Bengsch et al., 2010), indicating that targeting additional inhibitory pathways may be required to completely reverse the dysfunction of exhausted CD8+ T cells during HCV infection.

The mechanisms causing exhaustion of HCV-specific CD8+ T cells are still incompletely understood. Factors that might contribute to CD8+ T-cell dysfunction are continuous antigen-triggering, the lack of CD4+ T cell help or the action of regulatory T cells or cytokines. These possibilities will be discussed in the following.

Lack of CD4+ help

While CD8+ T cells are considered the major effector cells against viral pathogens, the successful elimination of HCV is probably highly dependent on sufficient CD4+ T-cell help. It has been demonstrated in the LCMV mouse model that CD4+ T-cell help is needed to sustain cytotoxic CD8+ T-cell responses during chronic viral infection (Matloubian et al., 1994). However, in chronic hepatitis C, CD4+ T-cell responses have been suggested to be weak or even absent and functionally impaired, for example because of secretion of low amounts of IL-2 (Semmo et al., 2005). Importantly, using de novo CD154 (CD40 ligand) expression in response to HCV antigens as a readout, we could recently show that virus-specific CD4+ T cells are not physically deleted, but only functionally impaired (Semmo et al., 2005). Clearly, this impaired function of critical CD4+ T-cell help might be a central determinant of CD8+ T-cell dysfunction.

Suppression by regulatory T cells

Growing evidence suggests that regulatory T cells play a significant role in the suppression of virus-specific T cells. For example, in chronically HCV-infected patients, CD4+CD25+ T cells have been found at a higher frequency compared with individuals with resolved HCV infection or healthy controls (Sugimoto et al., 2003; Cabrera et al., 2004; Boettler et al., 2005). These regulatory T cells suppress the proliferation as well as IFN-γ secretion of virus-specific CD8+ T cells in vitro. The suppression by CD4+CD25+ T cells was shown to depend on cell–cell contact (Sugimoto et al., 2003) but independent from suppressive cytokines such as IL-10 and TGF-β in some (Boettler et al., 2005; Rushbrook et al., 2005), but not all studies (Cabrera et al., 2004). Interestingly, the suppression was not restricted to HCV-specific CD8+ T cells, but also included CD8+ T cells specific for other viruses, such as EBV or influenza (Boettler et al., 2005; Rushbrook et al., 2005). However, specificity in vivo might be mediated by the enrichment of CD4+CD25+ T cells in the liver (Ward et al., 2007) where they might limit immunopathology in the chronic phase of HCV infection by blocking virus-specific CD8+ T cells by direct cell–cell contact (Franceschini et al., 2009).

Of note, functional FoxP3+ CD4+ (Smyk-Pearson et al., 2008) and HCV-specific FoxP3+ CD4+ T cells (Heeg et al., 2009) have been identified during the course of acute HCV infection. However, no specific correlation with the outcome of infection could be established in these studies.

Another type of regulatory T cells in HCV infection are virus-specific regulatory CD8+ T cells that express high levels of IL-10. These regulatory T cells have been detected in the liver of HCV-infected individuals (Abel et al., 2006) and their suppression of virus-specific CD8+ effector T cells could be blocked by neutralizing IL-10 antibodies (Accapezzato et al., 2004). Indeed, the blockade of IL-10 resulted in a stronger expansion of virus-specific CD8+ T cells supporting a biological and active role of IL-10 in chronic HCV infection. It is also important to note that myeloid suppressor cells that can be induced by HCV might also contribute to CD8+ T-cell dysfunction, for example through the production of reactive oxygen species (Tacke et al., 2011).

Another important factor contributing to CD8+ T-cell dysfunction is the specific micro-environment and
architecture of the liver. Indeed, recent research has documented multiple mechanisms by which immune responses in the liver are biased toward tolerance. For example, liver dendritic cell subsets, but also diverse subsets of unconventional antigen-presenting cells, such as liver sinusoidal endothelial cells or hepatic stellate cells, may play an important role in inducing immune suppression (reviewed in Crispe, 2011). It is also interesting to note that hepatocytes can function as antigen-presenting cells and can activate CD8+ T cells. However, this seems to result in effector cells that are not fully functional although this has not been shown to be the case in HCV infection.

Taken together, these results suggest that different mechanisms operate HCV-specific CD8+ T-cell dysfunction (Fig. 3). However, the relative contribution of each of these different pathways needs to be clarified in future studies.

Summary

As with most viral infections, HCV induces antiviral defense responses, but in the majority of infected individuals, production of type I IFNs and induction of a large panel of ISGs fails to keep this virus in check. Strikingly, and most distressing for the patients, even therapeutic administration of IFN-α is only fully efficacious in approximately one half of cases. The underlying reasons are only partly understood and many controversies prevail in the field. One might assume that HCV devised strategies to counteract the early innate antiviral defense and the cleavage of MAVS by the NS3/4A protease is one example supporting this notion. However, with respect to the ‘effector phase’ of the innate response, we note that HCV is sensitive to the IFN-induced antiviral status not only in cell culture, but also in vivo. It is therefore tempting to speculate that IFN-induced suppression of viral replication might even promote persistence. This could be due to, for example the IFN-mediated reduction of viral antigen and RNA load, thus attenuating innate immunity, but eventually also causing substantial defects of subsequent adaptive immune responses against HCV. The latter play a central role in the outcome of infection with HCV-specific CD8+ T cells inhibiting HCV replication by cytolytic and non-cytolytic effector mechanisms. Yet, in the majority of cases, CD8+ T cells fail to eliminate the virus likely due to the emergence of viral escape mutations and impairments in CD8+ T-cell effector functions. A better understanding of these mechanisms is required for the development of therapeutic and especially prophylactic approaches. Even though directly acting antiviral drugs will reduce the burden of hepatitis C, the majority of countries with high HCV prevalence might only partly profit from this new therapy. Eradication of the virus will thus only be possible by immuno-prophylactic approaches for which detailed knowledge of the mechanisms underlying failure to control infection with this insidious pathogen is needed.

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