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Review

A target on the move: Innate and adaptive immune escape strategies of hepatitis C virus

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Abstract

Obligate intracellular parasites such as the hepatitis C virus (HCV) have to cope intensively with immune responses in order to establish persistent infection. Powerful antiviral mechanisms of the host act on several levels. The innate immune response is able to slow down viral replication and activate cytokines which trigger the synthesis of antiviral proteins. The adaptive immune system neutralizes virus particles and destroys infected cells. Viruses have therefore developed a number of countermeasures to stay moving targets for the immune system. Here, we attempt to summarize the current state of research regarding innate and adaptive immune responses against HCV and the different escape strategies evolved by this virus.

Keywords: Hepatitis C virus; Innate immunity; Interferon system; Adaptive immunity; Escape mechanisms

Contents

1. Hepatitis C virus: gene products and replication cycle

Hepatitis C virus (HCV) is an enveloped virus with a positive-strand RNA genome of ca. 9.6 kb in length. It is classified into the genus Hepacivirus of the *Flaviviridae*. The genome contains one long open reading frame, encoding a polyprotein of approximately 3000 amino acids length, which is flanked by nontranslated regions (NTRs, Fig. 1). In addition to the polyprotein, the expression of another HCV protein with yet unknown function has recently been described, the so-called F-protein, which is generated by ribosomal frameshifting (Walewski et al., 2001; Xu et al., 2001). The 5′ NTR mediates translation of the polyprotein by an internal ribosome entry site (IRES) (Tsukiyama-Kohara et al., 1992) and has as well as the 3′ NTR an important role for viral RNA replication (Friebe and Bartenschlager, 2002; Friebe et al., 2001; Kolykhalov et al.,

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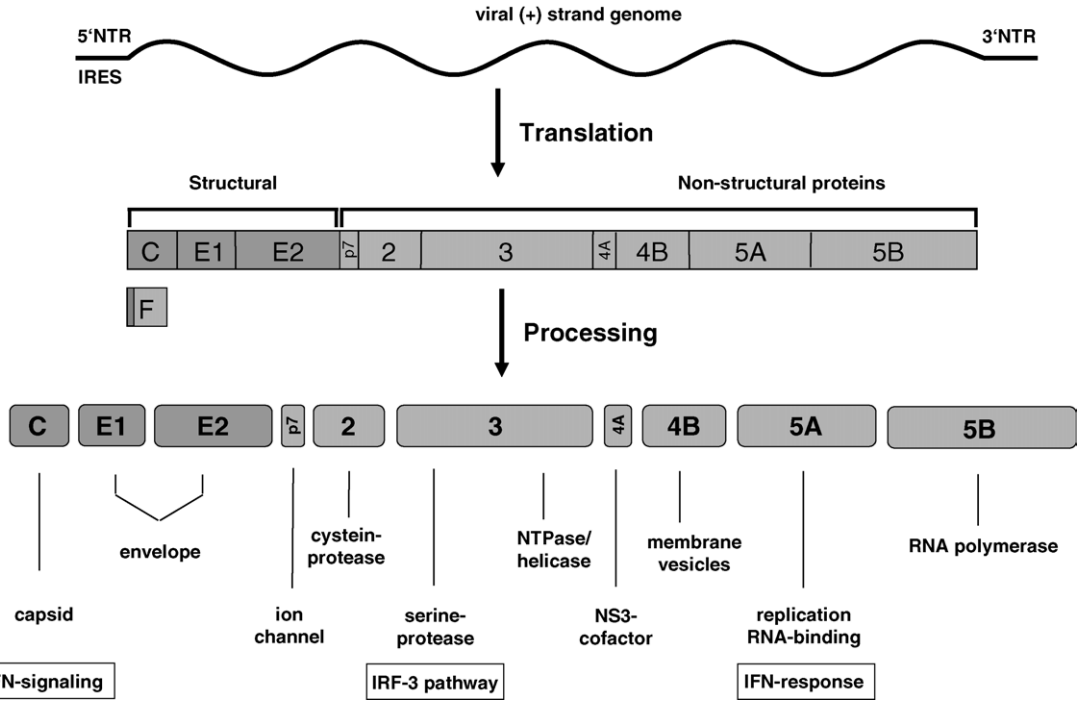

Fig. 1. Genomic organization of HCV. A schematic representation of the HCV genome with the 5′- and 3′-nontranslated regions (NTRs) is shown in the top, the translation products in the middle and the processed proteins with their known functions below. Interference of HCV proteins with certain pathways of the innate immune responses, which will be discussed in greater detail in this review is indicated at the bottom of the figure.

2000; Yi and Lemon, 2003a,b). The polyprotein is co- and post-translationally cleaved by viral and host-cell proteases into its functional subunits core (C), envelope protein 1 (E1), E2, p7, nonstructural protein 2 (NS2), NS3, NS4A, NS4B, NS5A and NS5B (reviewed in Reed and Rice, 2000). Core, E1 and E2 are the major constituents of the virus particle. Recent data imply that p7 might belong to the growing family of viral proteins which enhance membrane permeability in order to promote virus budding, the so-called viroporins (Griffin et al., 2003; Pavlovic et al., 2003). NS2 and the amino-terminus of NS3 comprise the NS2-3 protease responsible for cleavage between NS2 and NS3 (Grakoui et al., 1993b; Hijikata et al., 1993). NS3 is a multifunctional protein, consisting of an amino-terminal protease domain required for processing of the NS3 to 5B region (Bartenschlager et al., 1993; Grakoui et al., 1993a) and a carboxyterminal helicase/nucleoside triphosphatase domain (Kim et al., 1995; Suzich et al., 1993). NS4A is a cofactor that activates the NS3 protease function by forming a heterodimer (Bartenschlager et al., 1995; Lin et al., 1995). The hydrophobic protein NS4B induces the formation of a cytoplasmic vesicular structure, designated membranous web that appears to contain the replication complex of HCV (Egger et al., 2002; Gosert et al., 2003). NS5A is a phosphoprotein which binds RNA (Huang et al., 2005) and seems to play an important role in viral replication since most of the cell culture-adaptive mutations described so far are located within the central region of NS5A (Blight et al., 2000; Guo et al., 2001; Krieger et al., 2001; Lohmann et al., 2003). NS5B is the RNA-dependent RNA polymerase of HCV (Behrens et al., 1996; Lohmann et al., 1997), the key enzyme for viral transcription and replication.

Studies on HCV replication have been hampered for a long time by the lack of efficient cell culture models. This has changed during the last years, first by the development of subgenomic replicons (Lohmann et al., 1999) and recently by the production of authentic infectious viral particles in cell culture (Lindenbach et al., 2005; Wakita et al., 2005; Zhong et al., 2005). Both models have dramatically increased our understanding of the HCV life cycle (Bartenschlager et al., 2004; Lindenbach and Rice, 2005). The virus enters the cell most likely via receptor-mediated endocytosis followed by release of the viral RNA genome into the cytoplasm. After translation and processing of the proteins, the viral nonstructural proteins NS3-5B constitute the replication complex in a vesicular membrane structure designated "membranous web" (Gosert et al., 2003). RNA replication starts with the synthesis of negative-strand RNA probably leading to a double-stranded RNA (dsRNA) which serves as a template for progeny positive-strand RNA molecules that either enter a new translation/replication cycle or which are packaged into viral particles by budding into the endoplasmic reticulum and released through the secretory pathway.

Due to the absence of a stable genomic intermediate, HCV must produce new viral RNA and proteins constantly to maintain persistence, always in risk of being detected by the hosts innate or adaptive immune system. Some aspects of viral replication might be attributed to these constraints: the overall rate of viral replication and antigen production in infected tissue seems to be relatively low compared to other viruses, making a direct demonstration of viral proteins and RNA difficult (Chang et al., 2003). RNA replication is highly error prone, due to the lack of a proofreading function of NS5B, which has generated

a remarkable genetic diversity. HCV is currently divided into six major genotypes with many subtypes differing up to 35% in their nucleotide sequence (Simmonds et al., 2005) and exists as a quasispecies swarm within every infected individual. Finally, the viral replication complex seems to form a very rigid membrane structure, which is highly resistant to proteases and nucleases in vitro (Miyanari et al., 2003; Quinkert et al., 2005), probably helping to hide and protect the dsRNA from detection by the innate immune system.

In addition to these "passive" evasion strategies, the viral proteins core, NS3/4A protease and NS5A are discussed to play an active role in interference with innate immunity (Fig. 1), which will be discussed in greater detail below.

2. Innate immune responses—the interferon system

The type I interferon system which mainly involves IFN-α and β is a powerful and universal intracellular defense system against viruses of all sorts. This is best illustrated in knockout mice which are unresponsive to IFN-α/β due to targeted deletions in the type I IFN receptor (Muller et al., 1994). These mice quickly succumb to viral infections although they have a regular adaptive immune system (reviewed in Weber et al., 2004). Likewise, humans with genetic defects in STAT-1, which is involved in the signaling cascade of the IFN system, die of viral disease at an early age (Dupuis et al., 2003).

2.1. Interferon induction

All nucleated cells of the mammalian body are able to synthesize and secrete type I IFNs in response to virus infection. Secreted IFNs are then recognized by neighbouring cells and cause them to express potent antiviral proteins (Samuel, 2001; Weber et al., 2004). As a result, virus multiplication is slowed down or even stopped, and the organism buys time for the establishment of an adaptive immune response.

Type I IFNs are classified according to their amino acid sequence and comprise a large number (at least 13) of IFN-α subtypes and a single IFN-β (Stark et al., 1998), as well as some additional family members (Roberts et al., 2003; van Pesch et al., 2004). Expression patterns, i.e. which IFNs will be synthesized at which time point, mostly depend on the particular cell type.

Fibroblasts secrete mainly IFN-β as an initial response to infection but switch to IFN-α during the subsequent amplification phase of the IFN response (Marie et al., 1998). By contrast, dendritic cells, which play an important role in immunosurveillance, directly secrete high levels of IFN-α subtypes (Colonna et al., 2002; Diebold et al., 2003).

On the molecular level, induction of type I IFN gene expression by the so-called "classical pathway" is best understood for IFN-β in fibroblasts (Fig. 2). In infected cells, a signaling chain is activated by dsRNA molecules which are generated as intermediates of viral transcription. Two intracellular RNA helicases, RIG-I (Yoneyama et al., 2004) and MDA5 (Andrejeva et al., 2004), act as sentinels for viral dsRNA (Kato et al., 2005; Yoneyama et al., 2005). Then, a recently discovered protein binds to RIG-I and MDA5 and mediates the signal to downstream factors. It is called either IPS-1 for "interferon-β-promoter stimulator 1" (Kawai et al., 2005), MAVS for "mitochondrial antiviral signaling" molecule (Seth et al., 2005), VISA for "virus-induced signaling adaptor" (Xu et al., 2005) or Cardif for "CARD adaptor inducing IFN-β" (Meylan et al., 2005). IPS-1/MAVS/VISA/Cardif activates two IκB kinase (IKK)-related kinases, IKKε and TANK-binding kinase-1 (TBK-1), which phosphorylate the transcription factor IRF-3 (Fitzgerald et al., 2003; Sharma et al., 2003). IRF-3 is a member of the IFN regulatory factor (IRF) family and plays a central role in the activation of the IFN-β promoter (Lin et al., 1998; Schafer et al., 1998; Wathelet et al., 1998; Weaver et al., 1998; Yoneyama et al., 1998). Phosphorylated IRF-3 homo-dimerizes and moves into the nucleus where it recruits the transcriptional coactivators p300 and CREB-binding protein (CBP) to initiate IFN-β mRNA synthesis (Hiscott et al., 1999; Suhara et al., 2002). This first-wave IFN triggers expression of a related factor, IRF-7, which in fibroblasts is only present in low amounts (Sato et al., 2000). IRF-7 can be activated the same way as IRF-3 (Iwamura et al., 2001; Smith et al., 2001; tenOever et al., 2004), leading to a positive-feedback loop that initiates the synthesis of several IFN-α subtypes as the second-wave IFNs (Marie et al., 1998; Sato et al., 1998). In addition, NF-κB and AP-1 are recruited in a dsRNA-dependent way (Chu et al., 1999; Yang et al., 1995). Together these transcription factors strongly upregulate IFN-β gene expression.

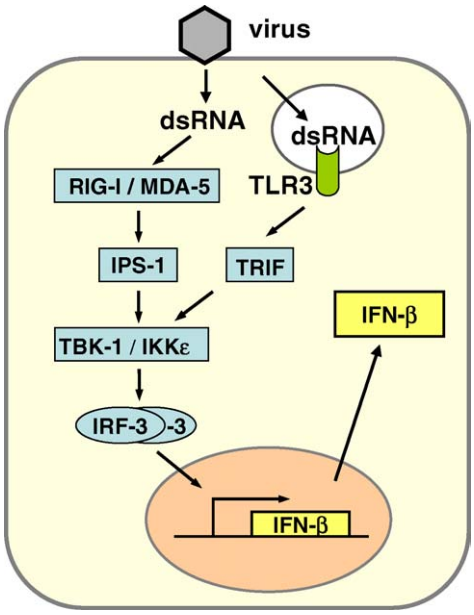

Fig. 2. Type I IFN gene expression. dsRNA, a characteristic by-product of viral replication, leads to activation of the transcription factor IRF-3. IRF-3 is phosphorylated by the kinases IKKε and TBK-1 which in turn are activated by the intracellular RNA-sensor proteins RIG-I and MDA5. IPS-1 (also termed MAVS/VISA/Cardif) serves as an adaptor protein connecting dsRNA sensing and IRF-3 phosphorylation. A second dsRNA signaling pathway involves endosomal TLR-3 and the adaptor protein TRIF.

Among the cells of the lymphatic system, myeloid dendritic cells (mDCs) (Diebold et al., 2003) and, most prominently, plasmacytoid dendritic cells (pDCs) (Colonna et al., 2002) are the

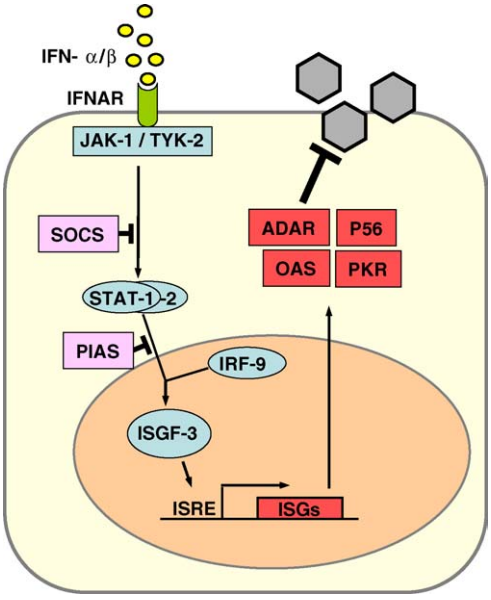

Fig. 3. Cellular response to IFNs. Newly synthesized IFN-α/β binds to its cognate receptor (IFNAR) and activates the expression of numerous IFN-stimulated genes (ISGs) via the JAK/STAT pathway. ADAR, P56, OAS and PKR are IFN-stimulated gene products with antiviral properties against HCV. The SOCS and PIAS proteins negatively regulate the IFN-induced signaling pathway at different stages.

main IFN producers. In addition to the classical, cytoplasmic pathway of IFN induction described above, pDCs sense the presence of viruses by the extracytoplasmic toll-like receptors (TLRs) (Beutler, 2004; Bowie and Haga, 2005). It is thought that TLRs serve as sensors for viral infection of phagocytosed cells (Schulz et al., 2005). Human pDCs mostly express TLR7 and TLR9 which recognize viral single-stranded (ss) RNA and dsDNA, respectively (Iwasaki and Medzhitov, 2004), whereas mDCs express TLR3 which responds to dsRNA (Alexopoulou et al., 2001). Upon activation, TLRs signal through different intracellular adaptor molecules such as MyD88 (TLR7 and 9) or TRIF (TLR3) to induce IFN transcription (Iwasaki and Medzhitov, 2004). Interestingly, DCs already contain high levels of IRF-7 (Kerkmann et al., 2003; Prakash et al., 2005), thus explaining their ability to rapidly produce high amounts of alpha-IFNs.

2.2. Interferon signaling

IFN-α/β subtypes all bind to and activate a common type I IFN receptor. It consists of two subunits (IFNAR-1 and IFNAR-2) and is present on virtually all host cells (Samuel, 2001; Stark et al., 1998). Binding of IFN-α/β leads to heterodimerization of the IFNAR subunits and to conformational changes in the intracellular parts of the receptor which activate the so-called JAK–STAT signaling pathway (Fig. 3). 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 (Levy and Darnell, 2002). Phosphorylated STAT-1 and STAT-2 recruit a third factor, IRF-9 (also called p48), to form a complex known as IFN-stimulated gene factor 3 (ISGF-3). The ISGF-3 heterotrimer translocates to the nucleus and binds to IFN-stimulated response elements (ISRE) in the promoter regions of IFN-stimulated genes (ISGs), thereby inducing their transcription.

Several specialized proteins serve as negative regulators and inhibitors of the JAK–STAT pathway. For example, the suppressor of cytokine signaling (SOCS) proteins specifically prevent STAT activation by binding to activated cytokine receptors, inhibiting the activity of JAKs, and targeting bound signaling proteins for proteasomal degradation (Kubo et al., 2003). Also, the 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 and Liu, 2005).

2.3. Interferon effector proteins with antiviral activity against HCV

IFN-α combined with ribavirin is the standard treatment for HCV infection, and its effect can be potentiated by co-adminitration of IFN-γ (Katayama et al., 2001; Okuse et al., 2005). IFN-α/β activates the expression of more than 300 IFN-stimulated genes (ISGs) which have antiviral, antiproliferative, and immunomodulatory functions (de Veer et al., 2001; Der et al., 1998). IFN-induced proteins include enzymes, transcription factors, cell surface glycoproteins, cytokines, chemokines and a large number of factors that need to be further characterized. Up to now, only a few antiviral proteins have been characterized in detail. Type I IFNs are known to be effective against HCV replicon systems (Frese et al., 2001; Guo et al., 2001), and several IFN-induced proteins have documented anti-HCV activity, namely protein kinase R (PKR) (Pflugheber et al., 2002), the RNA-specific adenosine deaminase 1 (ADAR 1) (Taylor et al., 2005), the 2′-5′ oligoadenylate synthetases (2–5 OAS)/RNaseL system (Guo et al., 2004), and P56 (Wang et al., 2003).

PKR, ADAR1 and 2–5 OAS are constitutively expressed in normal cells in a latent, inactive form. Basal mRNA levels are upregulated by IFN-α/β and these enzymes 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 (Williams, 1999). As a consequence, translation of cellular and viral mRNAs is blocked. ADAR 1 catalyzes the deamination of adenosine on target dsRNAs to yield inosine. As a result the secondary structure is destabilized due to a change from an AU base pair to the less stable IU base pair and mutations accumulate within the viral genome (Samuel, 2001). The 2–5 OAS catalyzes the synthesis of short 2′–5′ oligoadenylates that activate the latent endoribonuclease RNaseL (Silverman, 1994). RNaseL, in turn, then degrades both viral and cellular RNAs, leading to viral inhibition (Zhou et al., 1997). P56 binds the eukaryotic initiation factor 3e (eIF3e) subunit of the eukaryotic translation initiation factor eIF3. It functions as an inhibitor of translation initiation at the level of eIF3 ternary complex formation and is likely to suppress viral RNA translation (Hui et al., 2003; Terenzi et al., 2005).

3. Innate immune responses—natural killer cells

Natural killer (NK) cells play an important role in the host defense against various pathogens.

They recognize infected cells in an antigen-independent manner, destroy them due to their cytotoxic activity, and rapidly produce large amounts of IFN-γ to activate a cellular adaptive immune response (Tosi, 2005). The activity of NK cells is regulated by a balance between stimulatory and inhibitory receptors (Lanier, 1998).

4. Adaptive immune responses

Adaptive immune responses are primarily composed of humoral immune responses (e.g., antibodies produced by B cells) and, most important in viral infections, cellular immune responses (e.g., $CD4^+$ and $CD8^+$ T cells). $CD4^+$ T cells recognize antigens presented by MHC class II molecules on the surface of professional antigen presenting cells (APCs). Subsequently, $CD4^+$ T cells perform multiple effector functions, including direct activation of macrophages and antigen-specific B cells as well as activation of $CD8^+$ T cells in a cytokine-dependent manner. $CD8^+$ T cells recognize antigens presented by MHC class I molecules on the surface of infected cells. Of note, it has been recently shown that HCV-specific $CD8^+$ T cells can also be primed by cross-presentation of HCV antigens by DC (Accapezzato et al., 2005; Barth et al., 2005). Subsequently, $CD8^+$ T cells perform different effector functions, such as the killing of infected target cells and the secretion of cytokines such as IFN-γ and TNF-α that can inhibit viral replication without killing the infected cell (Guidotti and Chisari, 2001). The latter reflects the curative potential of $CD8^+$ T cells that were previously thought to be merely cytotoxic.

HCV infection results in antibody production to various HCV proteins in nearly all immunocompetent patients. Indeed, HCV-specific antibodies are usually detectable approximately 7–8 weeks after HCV infection. However, the role of antibodies in protection has been questioned since they do not prevent reinfection of immune chimpanzees or humans (Farci et al., 1992; Lai et al., 1994) and since they do not correlate with a favorable outcome (Chen et al., 1999). Important new insights about the potential role of anti-HCV antibodies have been forthcoming from recent studies using infectious lentiviral pseudotype particles bearing native HCV envelope glycoproteins (Bartosch et al., 2003; Logvinoff et al., 2004; Meunier et al., 2005). Indeed, cross-viral genotype neutralization of HCV by antibodies from chronically HCV infected persons has been demonstrated although these antibodies are rare in persons with resolved HCV infection (Bartosch et al., 2003; Logvinoff et al., 2004; Meunier et al., 2005). Thus, these results do not support a major role of neutralizing antibodies in the outcome of HCV infection.

In contrast, there is strong evidence for an important role of both virus-specific $CD4^+$ and $CD8^+$ T cells in viral control as well as liver injury (Bowen and Walker, 2005a; Neumann-Haefelin et al., 2005). Initial studies of acutely HCV-infected patients revealed that a strong, multispecific and sustained HCV-specific $CD4^+$ T cell response is associated with a self-limited course of infection (Diepolder et al., 1995; Gerlach et al., 1999; Missale et al., 1996). The $CD4^+$ T cell repertoire has been shown to be broad and to include dominant and highly promiscuous epitopes (Gerlach et al., 2005; Schulze Zur Wiesch et al., 2005). The virus-specific $CD8^+$ T cell response in acute resolving HCV infection is also vigorous and multispecific, targeting up to eight to twelve epitopes (Gruner et al., 2000; Lechner et al., 2000b). Important additional information came from the analysis of the T cell response in a health care worker after accidental needlestick exposure that led to HCV infection (Thimme et al., 2001). In this subject, the emergence of virus-specific $CD8^+$ T cells on week 7 after exposure was temporally associated with the onset of considerable liver disease (e.g., elevated liver enzymes) but no substantial decrease of viral titer. Importantly, in this early phase, the virus-specific $CD8^+$ T cells were not capable to produce IFN-γ, a property also referred to as stunned (Lechner et al., 2000b). In a later phase, $CD8^+$ T cells recovered their ability to produce IFN-γ, coinciding with a 5 log drop in viremia and resolution of liver disease. This implies that virus-specific $CD8^+$ T cells might contribute to viral clearance by cytolytic as well as non-cytolytic mechanisms that overlap in the course of acute infection. The important role of IFN-γ in viral clearance is further supported by findings in the HCV replicon model (Frese et al., 2002). In addition, genomic analysis of acutely infected chimpanzees revealed that transient or sustained viral clearance was associated with the up-regulation of IFN-γ induced genes in the liver (Bigger et al., 2001; Su et al., 2002).

Studies in experimentally HCV-infected chimpanzees showed that virus-specific $CD4^+$ and $CD8^+$ T cells accumulate in the liver about 8–14 weeks after infection and that this coincides with viral clearance and liver disease (Cooper et al., 1999; Thimme et al., 2002). The relative contribution of $CD4^+$ and $CD8^+$ T cell responses to viral clearance in acutely HCV-infected patients has not been fully defined. However, depletion studies in chimpanzees have demonstrated a crucial role for both, $CD4^+$ and $CD8^+$ T cells, in mediating protective immunity (Grakoui et al., 2003; Shoukry et al., 2003).

Importantly, even after HCV clearance, virus-specific $CD4^+$ and $CD8^+$ T cell responses remain detectable. In a cohort of women accidentally exposed to the same HCV strain, HCV-specific $CD4^+$ and $CD8^+$ T cells were still detectable two decades after recovery, whereas circulating HCV-specific antibodies were undetectable in several of these patients (Takaki et al., 2000).

5. Viral evasion strategies

Being a persistently infecting virus, HCV was forced to evolve sophisticated escape strategies for both the innate and the adaptive immune system. These countermeasures appear to be quite efficient, since 85% of the HCV-infected patients develop a chronic infection, and up to 60% of those patients do not respond to IFN therapy or experience a relapse when therapy is stopped (Pawlotsky, 2003). Furthermore, in patients as well as chimpanzees, multiple episodes of acute HCV infection have been

reported (Farci et al., 1992; Lai et al., 1994; Prince et al., 1992), indicating the absence of sterilizing immunity. Although some studies have described protective immune responses associated with reductions in viremia and duration of infection, these results nevertheless suggest the existence of viral evasion strategies to escape even pre-existing immune responses.

5.1. Evasion from innate immune responses

HCV is astonishingly efficient in disturbing the IFN response at multiple levels (Gale and Foy, 2005). With respect to IFN induction, it was recently discovered that the NS3/4A protease specifically cleaves IPS-1/MAVS/VISA/Cardif (Meylan et al., 2005) as well as TRIF (Breiman et al., 2005; Li et al., 2005). Since both these adaptor proteins are important for IFN induction via the classical intracellular pathway (IPS-1) and the TLR3-driven endosomal pathway (TRIF), NS3/4A is the key factor of HCV to disturb IRF-3 activation (Foy et al., 2003) which would otherwise result in IFN gene transcription. In addition, NS3 directly interacts with TBK1 to inhibit its association with IRF-3 and its activation (Otsuka et al., 2005).

With respect to the IFN response, it was shown that expression of the full-length virus genome or the core protein suppresses IFN signal transduction (Heim et al., 1999; Melen et al., 2004). Most likely, this is due to an up-regulation of protein phosphatase 2A, resulting in association of STAT1 with its inhibitor PIAS1 (Duong et al., 2004). Moreover, for the core protein it was shown that it interferes with the JAK/STAT pathway (de Lucas et al., 2005) and is able to activate the JAK–STAT signaling inhibitor SOCS-3 (Bode et al., 2003), further contributing to the HCV-induced block of IFN signaling.

HCV also directly counteracts the antiviral IFN response. The NS5A protein, which confers a multitude of functions in virus replication (Macdonald and Harris, 2004), also plays a key role in escape from the antiviral action of IFN. A stretch of 40 amino acids on NS5A, termed the IFN sensitivity region (ISDR), was correlated with responsiveness to IFN therapy (Enomoto et al., 1995, 1996; Witherell and Beineke, 2001). Moreover, NS5A was shown to directly bind to and repress PKR, and this interaction involved the ISDR (Gale et al., 1998). However, other groups did not find a connection between viral IFN susceptibility and a particular ISDR sequence (Aizaki et al., 2000; Aus dem Siepen et al., 2005; Paterson et al., 1999), and PKR activity was not affected by expression of the HCV genome (Francois et al., 2000) or NS5A (Podevin et al., 2001), although NS5A clearly reduced the antiviral effects of IFN (Podevin et al., 2001). A possible solution of this discrepancy could be that ISDR sequence variations affect the efficiency of HCV replication (Appel et al., 2005; Blight et al., 2000). Thus, the correlation between particular ISDR sequences and IFN sensitivity could be caused by differences in HCV replication strength. In addition, NS5A induces IL-8, a chemokine which inhibits the antiviral actions of IFN (Polyak et al., 2001a). Elevated IL-8 levels were indeed detected in the sera of IFN non-responders (Polyak et al., 2001b).

NS5A also interferes with the 2–5 OAS/RNaseL pathway by binding to 2–5 OAS (Taguchi et al., 2004). Furthermore, the HCV genome sequences of IFN resistant strains have fewer RNase L recognition sites than those of more IFN-sensitive ones (Han and Barton, 2002), thus allowing escape from nucleolytic cleavage (Han and Barton, 2002). PKR activity is also modified by the IRES of HCV (Vyas et al., 2003) and the E2 protein (Taylor et al., 1999).

Interestingly, HCV has also evolved strategies to evade the NK cell response. An important role for NK cells in early HCV infection has recently been suggested by Khakoo et al. (2004) who showed that genes encoding the inbibitory NK cell receptor KIR2DL3 and its human leukocyte antigen C ligand group 1 directly influence resolution of HCV. This has led to the hypothesis that the activation threshold of NK cells might be lower in these patients, which might support HCV clearance. It has also been shown that the envelope protein E2 crosslinks the HCV receptor CD81, thereby inhibiting cytotoxicity and IFN-γ production by NK cells (Crotta et al., 2002; Tseng and Klimpel, 2002). Of note, it has been recently demonstrated that NK cells from HCV-infected patients are impaired in their capacity to activate dendritic cells due to a overexpression of the receptor CD94-NK2A and production of interleukin 10 and transforming growth factor-β (Jinushi et al., 2004). Finally, Meier et al. (2005) recently described significantly reduced numbers and functional impairements of NK cells in chronically HCV infected patients.

5.2. Evasion from adaptive immune responses

The mechanisms responsible for the evasion of HCV from the adaptive immune response are still only partially understood. Various mechanisms of virus-specific T cell failure leading to HCV persistence have been suggested (Fig. 4), but so far most of them are poorly defined (Bowen and Walker, 2005a; Neumann-Haefelin et al., 2005). A primary failure to induce a T cell response or exhaustion of an initially vigorous response are two important predictors of viral persistence (Bowen and Walker, 2005a; Neumann-Haefelin et al., 2005). Indeed, several studies have shown that HCV-infected patients who develop chronic infection typically have only weak, oligo-/mono-specific or no virus-specific $CD4^+$ and $CD8^+$ T cell responses in the acute and chronic phase of infection (Bowen and Walker, 2005a; Lauer et al., 2004; Neumann-Haefelin et al., 2005). In addition, the direct loss (exhaustion) of HCV-specific $CD4^+$ T and $CD8^+$ cell responses in acute hepatitis has also been demonstrated in acutely infected patients that transiently controlled the virus but subsequently progressed to viral persistence (Gerlach et al., 1999; Lechner et al., 2000a). The mechanisms responsible for primary T cell failure or T cell exhaustion are thus far unclear. It has been suggested, for example, that antigen presentation by DCs and macrophages might be impaired in HCV infection (Bain et al., 2001; Lee et al., 2001; Sarobe et al., 2002), thus resulting in ineffective priming of T cells or impaired maintenance of antigen-experienced T cells. However, other studies have not been able to confirm the dysfunctions of the antigen-presenting cells (Longman et al., 2004). Thus, it is still unclear to which degree DC dysfunction contributes to viral persistence. Another explanation for T cell exhaustion might be the deletion of virus-specific T cells in the presence of continuous high viral load as has been demonstrated for lymphocytic choriomeningitis

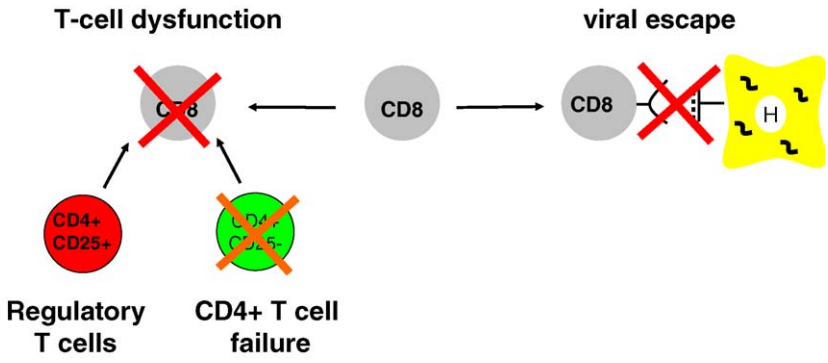

Fig. 4. Failure of virus-specific $CD8^+$ T cells. Viral amino acid substitutions within recognized epitopes inhibit $CD8^+$ T cell recognition of infected hepatocytes (H). Dysfunction of virus-specific $CD8^+$ T cells can either be caused by a lack of sufficient $CD4^+$ T cell help or the suppressive actions of regulatory T cells.

virus (LCMV) infection in adult mice (Moskophidis et al., 1993) or the rapid induction of activation induced cell death, e.g., in the liver (Nuti et al., 1998). In this context, it is interesting to note that in some chronically infected chimpanzees, virus-specific T cells were detectable in the blood but not in the liver (Thimme et al., 2002), supporting the concept of rapid loss of HCV-specific T cells from the liver. However, it is also possible that a failure of T cells to home to the infected organ is responsible for this observation.

Mutational escape from the adaptive immune response has been suggested as one of the major viral evasion strategies. HCV replicates at the enormous rate of about 10^{12} virions per day by the action of its RNA-dependent RNA polymerase that lacks a proofreading function, favoring Darwinian selection of variant viruses by humoral and cellular immune responses (Bowen and Walker, 2005b). Indeed, infection outcome in humans was predicted by sequence changes in the hypervariable region of the E2 envelope glycoprotein, a major target of the antibody response, that occurred at the time of antibody seroconversion (Farci et al., 2000). Thus, these sequence changes have been suggested to represent escape mutations within possible B cell epitopes.

Viral amino acid substitutions that inhibit HCV-specific T cell recognition have initially been observed in chronically HCV-infected patients (Chang et al., 1997; Frasca et al., 1999) and chimpanzees (Weiner et al., 1995). Interestingly, escape mutations detected in chronically infected patients did not diversify further during several years of follow-up (Chang et al., 1997). Consequently, it was suggested that T cell escape occurs early during infection. This hypothesis has been subsequently confirmed by an analysis of the early T cell-virus interactions in acutely infected chimpanzees (Erickson et al., 2001) and humans (Bowen and Walker, 2005b; Cox et al., 2005; Tester et al., 2005; Timm et al., 2004). Population based approaches have also further provided support for T cell driven HCV evolution (Ray et al., 2005; Timm et al., 2004). Interestingly, variant-specific virus-specific T cell responses are usually undetectable in these patients. The failure to generate variant-specific $CD8^+$ T cell responses can be explained by the lack of sufficient $CD4^+$ T cell help or a variant epitope-mediated expansion of wild-type-specific $CD8^+$ T cells, a phenomenon known as original antigenic sin (Klenerman and Zinkernagel, 1998). It is important to note, however, that the importance of viral escape mutations for the development of HCV persistence is still not completely understood. Indeed, viral escape occurs typically in the presence of a CTL response that is focussed on a single viral epitope. This type of T cell response is unusual, however, during acute HCV infection. Accordingly, the loss of a single epitope would probably not be sufficient for the survival of viral escape mutants. In addition, most studies have clearly shown that the development of viral escape mutations is not universal. For example, viral clearance can occur with minimal epitope variation prior to resolution and studies in chronically HCV-infected chimpanzees and humans have shown that not all epitopes restricted by class I alleles undergo variations to produce escape mutations (Chang et al., 1997; Erickson et al., 2001). The virological (e.g., viral fitness cost) and immunological (e.g., genetically restricted T cell repertoire or TCR diversity) factors that determine the occurrence of escape mutations in HCV infection are currently not well defined (Bowen and Walker, 2005b; McKiernan et al., 2004; Meyer-Olson et al., 2004).

Another important possible mechanism of immune evasion is functional anergy of virus-specific T cells. Indeed, several studies using the tetramer technique have shown that dysfunction of $CD8^+$ T cells occurs in acute as well as chronic HCV infection (Gruener et al., 2001; Lechner et al., 2000a; Thimme et al., 2001; Wedemeyer et al., 2002). Indeed, HCV-specific $CD8^+$ T cells may be impaired in their proliferative capacity, cytotoxicity, and ability to secrete TNF-α and IFN-γ upon stimulation, referred to as stunned phenotype. Interestingly, T cell dysfunction was observed in the early course of acute HCV infection in all patients irrespective of virological outcome. In patients with a self-limited course of infection, however, the recovery of $CD8^+$ T cell function was temporally associated with a sharp decline of viremia and resolution of disease (Lechner et al., 2000a; Thimme et al., 2001). In contrast, $CD8^+$ T cell function remained suppressed in patients who progressed to chronic infection (Urbani et al., 2002). Interestingly, the impaired effector functions of HCV-specific $CD8^+$ T cells are also associated with an immature differentiation phenotype of these HCV-specific $CD8^+$ T cells (Appay et al., 2002). Indeed, whereas CMV- and influenza virus-specific $CD8^+$ T cells show a highly differentiated phenotype ($CCR7^-CD45RA^+$, $CD28^-CD27^-$), HCV-specific $CD8^+$ T cells express less differentiated phenotypes (Appay et al., 2002; Champagne et al., 2001; Urbani et

al., 2002). HCV-specific $CD8^+$ T cells are mostly $CD28^+$ and/or $CD27^+$, indicating that they remain in an early differentiation stage. Of note, in chronically HCV-infected patients, not only HCV-specific, but also CMV-specific $CD8^+$ T cells have been shown to express a less mature phenotype, suggesting that the pervasive influence of HCV on $CD8^+$ T cells does not only affect HCV-specific T cells (Lucas et al., 2004). However, CMV-specific $CD8^+$ T cells in these patients displayed no functional impairment in vitro and immunodeficiency resulting in CMV reactivation has never been observed in chronic HCV infection.

Of note, HCV-specific $CD8^+$ T cells are also impaired in their antiviral effector functions in the infected organ, the liver. Indeed, a recent study showed that intrahepatic HCV, but not influenza virus-specific $CD8^+$ T cells are impaired in their ability to secrete IFN-γ despite the accumulation of these cells in the liver (Spangenberg et al., 2005). It is tempting to speculate that the different mechanisms of $CD8^+$ T cell failure are a direct result of weak and dysfunctional virus-specific $CD4^+$ T cell help that is common in persistent HCV infection (Day et al., 2003; Semmo et al., 2005; Ulsenheimer et al., 2003). The important role of $CD4^+$ T cells is further strengthened by a recent $CD4^+$ T cell depletion study in the chimpanzee model where incomplete control of HCV replication by memory $CD8^+$ T cell responses in the absence of $CD4^+$ T cells was associated with the emergence of viral escape mutations in class I epitopes and a failure to resolve the infection (Grakoui et al., 2003). Growing evidence also suggests an important role of regulatory $CD4^+CD25^+$ T cells in the suppression of HCV-specific $CD8^+$ T cells. Indeed, these cells have been found to be enriched in chronically HCV-infected patients when compared with recovered or uninfected subjects and in vitro depletion studies and co-culture experiments revealed that peptide-specific IFN-γ production as well as proliferation of HCV-specific $CD8^+$ T cells were inhibited by $CD4^+CD25^+$ T cells in a dose dependent manner and by direct-cell–cell contact (Boettler et al., 2005; Cabrera et al., 2004; Rushbrook et al., 2005; Sugimoto et al., 2003). In addition, intrahepatic IL-10 producing regulatory $CD8^+$ T cells have also been recently described (Accapezzato et al., 2004). Although these results point to an important role of regulatory T cells in HCV persistence, several important questions, such as antigen specificity and liver compartmentalization of regulatory T cells still need to be addressed.

In summary, several different mechanisms seem to contribute to the failure of the adaptive immune response with mutational escape and functional anergy playing probably the most important role. As shown in Fig. 4, a lack of $CD4^+$ T cell help and/or the action of regulatory T cells contributes to the failure of the virus-specific $CD8^+$ T cells. Clearly, a better understanding of the evasion strategies determining the outcome of HCV infection is required for the development of effective vaccines.

6. Concluding remarks

Despite the limited size of its genome, HCV has evolved an astonishing array of sophisticated survival strategies. It is not surprising, therefore, that HCV is so effective in spreading in human populations, that an efficient vaccine is still not in sight and that IFN therapy is not always effective. However, our rapidly increasing knowledge about HCV immune escape will certainly lead to a significant improvement of both prevention and therapy of hepatitis C.

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