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REVIEW

Hepatitis C Virus-Mediated Modulation of Cellular Immunity

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Abstract The hepatitis C virus (HCV) is a major cause of chronic liver disease globally. A chronic infection can result in liver fibrosis, liver cirrhosis, hepatocellular carcinoma and liver failure in a significant ratio of the patients. About 170 million people are currently infected with HCV. Since 80 % of the infected patients develop a chronic infection, HCV has evolved sophisticated escape strategies to evade both the innate and the adaptive immune system. Thus, chronic hepatitis C is characterized by perturbations in the number, subset composition and/or functionality of natural killer cells, natural killer T cells, dendritic cells, macrophages and T cells. The balance between HCV-induced immune evasion and the antiviral immune response results in chronic liver inflammation and consequent immune-mediated liver injury. This review summarizes our current understanding of the HCV-mediated interference with cellular immunity and of the factors resulting in HCV persistence. A profound knowledge about the intrinsic properties of HCV and its effects on intrahepatic immunity is essential to be able to design effective immunotherapies against HCV such as therapeutic HCV vaccines.

Keywords HCV · Kupffer cells · Dendritic cells · NK cells · NKT cells · Adaptive immune response

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Abbreviations

DAA	Directly acting antiviral
DC	Dendritic cell
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
IFN	Interferon
IL	Interleukin
KIR	Killer cell immunoglobulin-like receptor
MHC	Major histocompatibility complex
NK cell	Natural killer cell
NKT cell	Natural killer T cell
NS	Non-structural
PBMC	Peripheral blood mononuclear cell
PD	Programmed death
TCR	T cell receptor
TGF	Tumor growth factor
Th	T-helper cell
TIM	T cell immunoglobulin domain and mucin domain protein
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cell

Introduction

With approximately 170 million people infected with hepatitis C virus (HCV) globally, HCV represents a significant health burden. An infection with HCV is characterized by a probability of 70–80 % to develop a chronic infection accompanied by liver inflammation which causes liver fibrosis, cirrhosis and an increased risk to develop hepatocellular carcinoma in 30 % of the cases (Lauer and Walker 2001; Liang et al. 2000; Poynard et al.

2003). Since the current treatment based on pegylated interferon (IFN)-α and ribavirin can cure only about 55 % of the treated patients and is associated with various side effects, more efficient therapy options with fewer side effects are necessary. The recently developed directly acting antiviral (DAA) compounds for HCV will be able to improve the treatment success especially for the difficult-to-treat patients infected with the HCV genotype 1, but will constitute in addition to the pegylated IFN-α and ribavirin-based therapy in the first years. Thus, the development of prophylactic and therapeutic vaccines is an urgent need. A better understanding of the HCV-mediated modulation of cellular immunity is a prerequisite for the design of an effective vaccine.

HCV is an enveloped, positive-sense, single-stranded RNA virus belonging to the genus Hepacivirus in the Flaviviridae family. There are six HCV genotypes which differ in their geographic distribution and their responsiveness to antiviral therapy. The 9.6-kb RNA genome of HCV consists of a long open reading frame that is translated to a single polyprotein of approximately 3,000 amino acids. This polyprotein is co- and posttranslationally cleaved into ten structural and non-structural (NS) proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Bode et al. 2009). As a persistent virus, HCV has evolved mechanisms both to utilize and control cellular molecules or pathways required for the viral life cycle and to evade elimination by innate and adaptive immunity. The present review summarizes recent findings focusing on our current understanding of the mechanisms allowing HCV to interfere with host antiviral cellular immunity.

Antiviral Innate Immunity

The liver contains a large macrophage population named Kupffer cells and an unusually high frequency of natural killer (NK) and NK T (NKT) cells. In addition, several types of dendritic cells (DCs) populate the liver. When HCV infects the liver, infected hepatocytes and/or other cells react by secreting type I IFNs. The source of type I IFNs is currently unclear in vivo, but in vitro experiments suggest that it may be produced by either hepatocytes or plasmacytoid DCs (pDCs) (Shin et al. 2006; Takahashi et al. 2010). Type I IFNs not only induce cell death of the infected hepatocytes and an antiviral state in the neighboring uninfected cells, but also activate innate immune cells such as Kupffer cells, DCs, NK cells and NKT cells (Bode et al. 2008). The activated innate immune cells multiply the antiviral response by releasing pro-inflammatory cytokines and chemokines, thus inducing the activation of liver-resident immune cells and the recruitment of immune cells from the periphery. DCs have a key function in bridging innate and adaptive immunity since DCs are able to migrate from the site of infection to lymphoid tissues and to prime naïve T cells by presentation of the viral antigen. This finally results in the induction of virus-specific T and B cell responses. Several lines of evidence indicate that HCV can interfere with the activation and action of innate immune cells.

HCV-Mediated Effects on Innate Immune Cells

HCV-Mediated Modulation of the NK Cell Response

NK cells are characterized by their ability to kill virally infected and tumor cells without major histocompatibility complex (MHC) restriction or prior sensitization. Their importance for liver immunology becomes evident by the fact that their frequency in the liver (30–50 % of the lymphocytes) is much higher than in the peripheral blood (5–20 %) (Corado et al. 1997). There are two main NK cell subsets, which are distinguished by the expression level of the surface receptors CD56 and CD16: $CD56^{dim}$ $CD16^{bright}$ and $CD56^{bright}$ $CD16^{dim}$. $CD56^{dim}$ $CD16^{bright}$ NK cells are regarded as the more mature subset and have a high cytotoxic potential by both degranulation of cytotoxic granules and activation of death receptors such as the Fas receptor and the tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor. The less mature $CD56^{bright}$ $CD16^{dim}$ NK cells have an immunomodulatory function and are able to secrete a variety of cytokines such as granulocyte–macrophage colony-stimulating factor, IFN-γ, interleukin (IL)-10, IL-13, tumor growth factor (TGF)-β and TNF-α (Andoniou et al. 2006).

NK cell function is regulated by the interplay of stimulatory and inhibitory receptors such as the killer cell immunoglobulin-like receptors (KIRs), lectin-like receptors (NKG2A-F) and natural cytotoxicity receptors (NKp30, NKp44 and NKp46). Interestingly, genetic studies could show that the combination of the gene for the inhibitory receptor KIR2DL3 and the gene for the group 1 human leukocyte antigen-C (HLA-C1) ligand are associated with both spontaneous HCV clearance and beneficial response to IFN-α/ribavirin treatment (Khakoo et al. 2004; Knapp et al. 2010; Vidal-Castineira et al. 2010). This gene combination may cause protective effects by conferring to NK cells the ability to respond faster to a viral infection (Ahlenstiel et al. 2008). Furthermore, patients homozygous for the HLA-E(R) allele were shown to be protected against chronic infection with the HCV genotypes 2 and 3 (Schulte et al. 2009).

In acute HCV infection, NK cells show an activated state characterized by increased expression of the activating receptor NKG2D and enhanced cytotoxicity with no

evidence of a suppressive effect of HCV on NK cell function (Amadei et al. 2010; Pelletier et al. 2010). In chronic HCV infection however, perturbations in the number, subset composition and functionality of NK cells have been found (Fig. 1). The frequency of NK cells was reduced in both the peripheral blood (Meier et al. 2005; Morishima et al. 2006; Nattermann et al. 2006) and the liver (Kawarabayashi et al. 2000) of chronic HCV patients. Furthermore, skewing of NK cell subset distribution toward increased numbers of the cytokine-producing $CD56^{bright}$ $CD16^{dim}$ population, relative to the cytotoxic $CD56^{dim}$ $CD16^{bright}$ subpopulation, was reported (Golden-Mason et al. 2008b; Lin et al. 2004; Morishima et al. 2006). This may be caused by defects in IL-15 production by DCs, since IL-15 is critical for NK cell development and maturation (Meier et al. 2005). HCV-induced interference with NK cell functionality becomes evident by changes in the cytokine profile of NK cells in chronic HCV patients. While IFN-γ release by NK cells is decreased (Ahlenstiel et al. 2010; Oliviero et al. 2009), the production of IL-10 and TGF-β is increased (De Maria et al. 2007; Jinushi et al. 2004). Thus, cytokine production by NK cells in chronic HCV infection is skewed toward secretion of Th2 type cytokines promoting an environment, which is more permissive for HCV (Fig. 1).

The function of NK cells in chronic HCV infection may be directly impaired by the binding of the HCV E2 protein to CD81, which has an inhibitory function on NK cells (Crotta et al. 2002; Tseng and Klimpel 2002). However, reports analyzing this effect by using HCV viral particles instead of recombinant E2 proteins have been contradictory so far (Crotta et al. 2010; Yoon et al. 2009). Furthermore, the HCV core protein is able to impair NK cell activity via p53-dependent upregulation of TAP1 and consequently MHC class I surface expression. Enhanced MHC class I expression on infected hepatocytes confers them resistance to NK cell-mediated killing (Herzer et al. 2003). Additionally, a peptide derived from HCV core (HCV core 35–44) stabilizes HLA-E surface expression by binding to HLA-E and thereby impairing NK cell cytotoxicity. This may be mediated by the interaction of HLA-E with the inhibitory NK cell receptor CD94/NKG2A (Nattermann et al. 2005).

Thus, dysfunction of NK cells is critically involved in the establishment of chronic HCV infection. In particular, when one takes into consideration that NK cells not only have direct functions in the eradication of infected hepatocytes but also influence the function of DCs and T cells. Increased expression of the inhibitory receptor CD94/NKG2A in combination with enhanced release of IL-10 and TGF-β by NK cells from HCV-infected patients results in reduced capacity to activate DCs (Jinushi et al. 2004). Furthermore, the increase in NK cell-mediated IL-10 and TGF-β production is skewing the Th1/Th2 balance toward a Th2 response favoring T cell exhaustion and HCV chronicity (Fig. 1).

Role of NKT Cells in HCV Infection

NKT cells are a unique subset of T lymphocytes that co-express T cell receptors (TCRs) and NK cell markers and have both immunoregulatory and effector functions (Taniguchi et al. 2003). They recognize glycolipids presented by MHC class 1b molecules (CD1d in mice and multiple CD1 isoforms in humans) and express either a highly restricted TCR repertoire (invariant NKT cells) or a diverse TCR repertoire (variant NKT cells). When stimulated, they can secrete cytokines (IL-4, IFN-γ and TNF-α), express

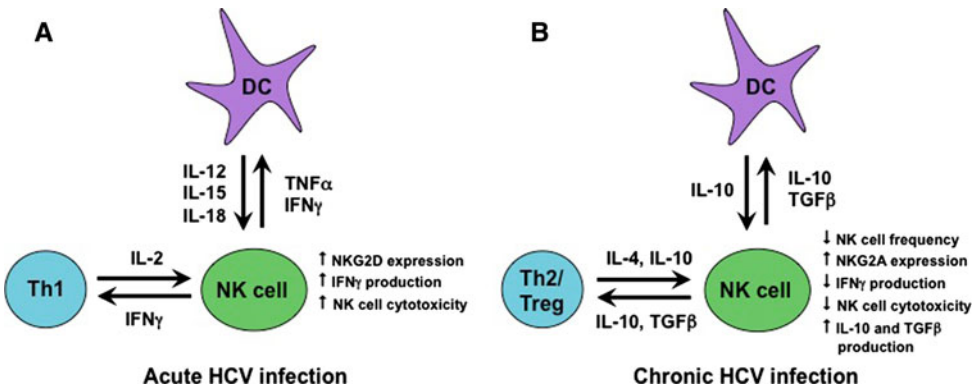

Fig. 1 NK cells in acute and chronic HCV infection. **a** In acute HCV infection, NK cells are activated (increased NKG2D expression) and are characterized by enhanced cytotoxicity and IFN-γ production. IFN-γ and TNF-α produced by NK cells prime a Th1 response and activate DCs which provide activating signals for NK cells by secreting IL-2, IL-12, IL-15 and IL-18. **b** In chronic HCV patients, NK cells are reduced in their frequency and functionality (reduced cytotoxicity and IFN-γ production, enhanced NKG2A expression). Instead, they produce IL-10 and TGF-β resulting in the induction of Th2 cells and Tregs in impaired DC activation and in further production of immunosuppressive cytokines such as IL-10

Fas ligand and activate other cell types such as dendritic and NK cells, which suggest that they may be involved in both clearance of virally infected cells and immune-mediated liver damage.

NKT cells are abundant in the liver comprising of about 30 % of the intrahepatic lymphocytes in mice and up to 50 % in humans (Geissmann et al. 2005; Norris et al. 1999). In chronic hepatitis C patients, decreased frequencies of intrahepatic NKT cells have been reported (Deignan et al. 2002; Kawarabayashi et al. 2000; Yamagiwa et al. 2008). Additionally, sustained response to IFN-α/ribavirin treatment is paralleled by a significant increase in the number of intrahepatic NKT cells (Yamagiwa et al. 2008). Thus, NKT cells may play a beneficial role in HCV clearance. In contrast, the number of activated NKT cells in the liver of chronic hepatitis C patients was shown to correlate with the degree of hepatocellular damage and the onset of fibrosis suggesting that NKT cells are also involved in the deleterious effects mediated by immune cells during chronic liver inflammation (de Lalla et al. 2004; Nuti et al. 1998).

Alteration of DC Functions by HCV

DCs play an essential role in the initiation of virus-specific T cell responses by taking up antigens, processing them and presenting antigen-derived peptides to T cells. After activation through antigen uptake and stimulation by inflammatory cytokines, DCs secrete chemokines, cytokines and IFNs resulting in the recruitment of inflammatory infiltrates to the sites of infection. Furthermore, they migrate to secondary lymphoid organs where they prime T cells and initiate the virus-specific T cell response. There are two major DC subsets in humans, the myeloid DCs (mDCs) and the plasmacytoid DCs (pDCs). While mDCs secrete mainly IL-10 and IL-12 and express Toll-like receptor (TLR)3 and TLR8, pDCs are characterized by IFN-α production and expression of TLR7 and TLR9.

Functional DCs seem to be of high importance to avoid HCV chronicity. Only patients who are able to increase the absolute number and percentage of circulating mDCs during acute hepatitis C are capable of eradicating the virus, while those who do not show any changes in mDCs numbers or frequencies develop viral persistence (Perrella et al. 2006a). Chronic HCV infection results in interference with both the number and the functionality of mDCs and pDCs (Fig. 2). The peripheral blood frequency of mDCs and pDCs is reduced in chronic hepatitis C patients (Kanto et al. 2004; Murakami et al. 2004; Wertheimer et al. 2004). Furthermore, mDCs and pDCs from these patients seem to be impaired in their maturation (Auffermann-Gretzinger et al. 2001; Mengshol et al. 2009).

Moreover, mDCs from HCV-infected patients are impaired in their abilities to stimulate allogeneic $CD4^+$ T cells and to produce IL-12, while their capacity to secrete IL-10 is increased, thus creating an immunosuppressive environment (Averill et al. 2007; Kanto et al. 2004; Murakami et al. 2004). Interestingly, mDCs from HCV-infected patients are not only characterized by an increase in their own IL-10 production but also by a profound ability to prime IL-10-producing T cells (Kanto et al. 2004). Since inhibitory receptors play an important role in the development of exhausted HCV-specific T cells, the expression of receptors with inhibitory function was also investigated on DCs. And indeed, programmed death (PD)-L1 expression on mDCs from chronic hepatitis C patients was shown to be increased and inversely correlated with their allostimulatory capacity (Dolganiuc et al. 2008; Shen et al. 2010) (Fig. 3). Additionally, mDCs from these patients trigger the proliferation of regulatory T cells (Tregs), which further leads to an impairment of the HCV-specific T cell response (Dolganiuc et al. 2008). The HCV core protein may be of main importance for the described effects on mDCs, since stimulation of mDCs with core resulted in inhibition of priming of antigen-specific $CD4^+$ and $CD8^+$ T cells and development of IL-10-producing Tregs (Zimmermann et al. 2008). The inhibitory effect of cell culture-derived HCV on TLR ligand-mediated mDC activation of naïve $CD4^+$ T cells was equally mediated mainly by core (Liang et al. 2009).

pDCs from HCV-infected patients have also been shown to have a reduced allostimulatory capacity (Kanto et al.

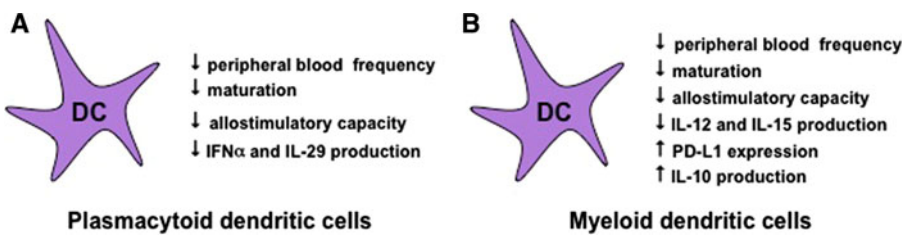

Fig. 2 Plasmacytoid and mDCs in chronic HCV infection. **a** In chronic HCV infection, plasmacytoid DCs are decreased in their frequency, their maturation and their allostimulatory capacity. Additionally, they are characterized by decreased production of IFN-α and IL-29. **b** In chronic HCV infection, mDCs are reduced in frequency, maturation and allostimulatory capacity. While their expression of PD-L1 and their production of IL-10 are increased, their secretion of IL-12 and IL-15 is impaired

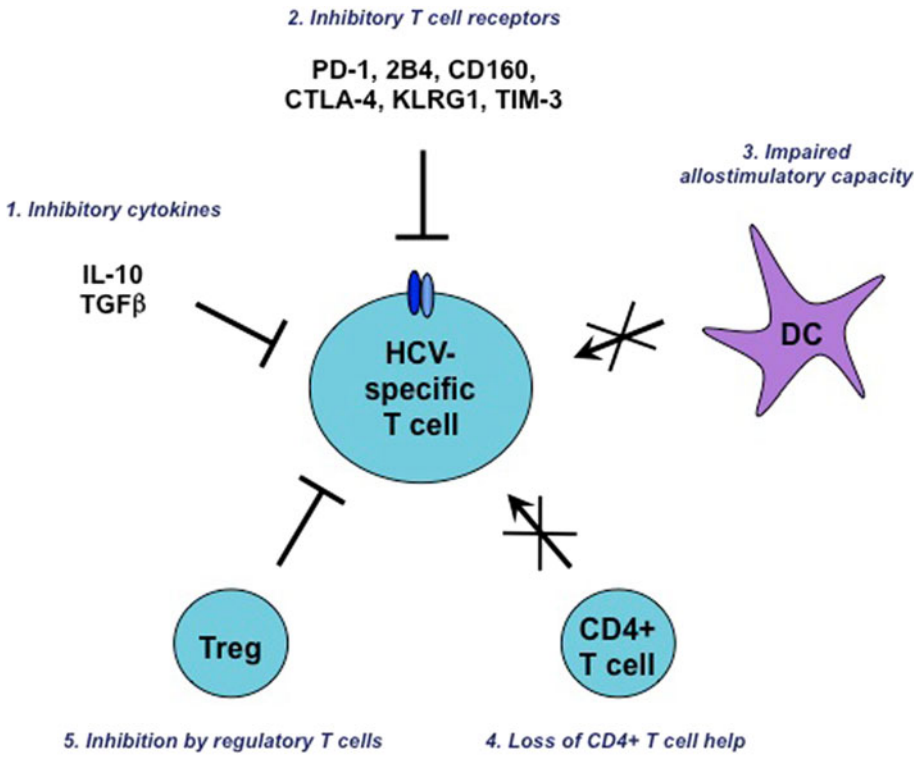

Fig. 3 Factors resulting in the exhaustion of HCV-specific T cells. Factors contributing to the development of HCV-specific T cell exhaustion are: *1*. inhibitory cytokines (such as IL-10 and TGF-β); *2*. inhibitory T cell receptors (such as PD-1, 2B4, CD160, CTLA-4, KLRG1 and TIM-3); *3*. impaired allostimulatory capacity of DCs; *4*. loss of $CD4^+$ T cell help; *5*. inhibition by Treg cells

2004) (Fig. 3). In addition, their ability to produce IFN-α is impaired (Dolganiuc et al. 2006; Kanto et al. 2004; Murakami et al. 2004). The HCV proteins core and NS3/4A seem to play a key role in the HCV-mediated modification of pDC function and frequency. The number of intrahepatic pDCs is significantly reduced in mice with liver-specific expression of NS3/4A (Frelin et al. 2006). Furthermore, HCV core and NS3 are able to activate monocytes to produce IL-10 and TNF-α, which result in inhibition of IFN-α secretion by pDCs and pDC apoptosis (Dolganiuc et al. 2003, 2006). Recently, it was shown that the level of IL-29 (IFN-λ1), which is mainly produced by DCs, was substantially lower in patients with chronic hepatitis C as compared to both healthy controls and patients with spontaneously resolved hepatitis. Interestingly, exposure of DCs to NS3 resulted in reduced IL-29 secretion in response to stimulation with poly I:C (Langhans et al. 2011). Besides, both core and NS3 have been shown to inhibit differentiation and allostimulatory capacity of immature DCs (Dolganiuc et al. 2003).

Influence of HCV on Intrahepatic Macrophages

The liver contains a large macrophage population named Kupffer cells, which act as both phagocytes and antigen-presenting cells. Hence, they are involved in the clearance of pathogen-derived particles and toxins and in the killing of pathogens and tumor cells but also contribute to tissue damage during chronic inflammation. In chronic HCV infection, most Kupffer cells are activated and express high levels of CD80, CD40 and MHC-II, thus acquiring the phenotype of professional antigen-presenting cells. Furthermore, activated Kupffer cells display a close contact with $CD4^+$ T cells and form Kupffer cell–T cell clusters (Burgio et al. 1998). Since they exhibit an activated phenotype, they fail to show homo- or hetero-tolerance to TLR ligands as the ones from controls or patients with non-alcoholic steatohepatitis (Dolganiuc et al. 2007). By expressing a variety of TLRs, Kupffer cells can easily sense viral pathogens and respond by secreting inflammatory cytokines such as TNF-α. In the context of HCV, the HCV proteins core and NS3 are able to induce TLR2/MyD88-dependently the secretion of IL-10 and TNF-α by monocytes/macrophages (Dolganiuc et al. 2003, 2004). In vivo, it has been shown that mice with liver-specific expression of NS3/4A are characterized by increased intrahepatic levels of CCL2 and TNF-α, which is paralleled by an enhanced intrahepatic number of macrophages. Thus, NS3/4A may induce a CCL2-mediated recruitment of macrophages to the liver resulting in increased TNF-α levels (Brenndorfer et al. 2010).

Since the expression of inhibitory receptors plays an important role in the induction of exhausted HCV-specific T cells, the expression of ligands of these receptors has also been investigated in Kupffer cells. Interestingly, galectin-9, the natural ligand for the inhibitory receptor T cell immunoglobulin domain and mucin domain protein (TIM)-3

is in the liver mainly expressed on Kupffer cells and its expression is significantly increased in patients with chronic HCV as compared to normal controls. Since galectin-9 has been shown to be involved in the expansion of Tregs, the contraction of $CD4^+$ effector T cells and the apoptosis of HCV-specific $CD8^+$ cells, Kupffer cell-derived galectin-9 seems to be of high importance for the suppression of the HCV-specific T cell response (Mengshol et al. 2010). Besides T cells, the inhibitory receptors PD-1 and TIM-3 are also expressed on macrophages. In chronic hepatitis C patients, PD-1 and TIM-3 were found to be overexpressed on monocytes/macrophages and to be associated with impairment in the production of IL-12 (Zhang et al. 2011a, b). Thus, Kupffer cells are involved in both the immunopathology associated with HCV by secreting high amounts of TNF-α and in the HCV-mediated interference with T cell immunity.

Antiviral Adaptive Immunity

The adaptive immune response is characterized by cellular and humoral effectors recognizing specific viral epitopes. T lymphocytes require epitope presentation by MHC molecules: $CD8^+$ cytotoxic T lymphocytes by MHC-I and $CD4^+$ Th lymphocytes by MHC-II molecules. $CD8^+$ T cells are responsible for the elimination of infected cells through perforin-mediated cytolysis or activation of the death receptor pathways. While Th1 cells activate $CD8^+$ T cells by secreting IFN-γ, IL-2 and TNF-α, Th2 cells are crucial for B cell activation and antibody secretion through the production of IL-4, IL-5, IL-6 and IL-13. When activated by antigen interaction and Th2 signals, B cells release antibodies, which are able to bind free virus and lyse infected cells. HCV infection of the liver results in production of IFN-β and IFN-α, which induce Kupffer cells to secrete MIP1α/CCL3, thereby recruiting NK cells. The recruited NK cells induce DC activation by cell–cell contact and production of IFN-γ and TNF-α. Priming of B and T cells in secondary lymphoid organs by activated mature DCs is necessary to initiate an effective antigen-specific adaptive immune response.

Humoral Immune Response During HCV Infection

Neutralizing antibodies produced by B cells are of importance for the control of many viral infections acting against both free virus and infected cells (Parren and Burton 2001). The binding of antibodies to free viral particles causes a loss of viral infectivity by inhibiting viral attachment or viral entry. Further, the Fc chain of antibodies mediates complement activation leading to opsonization of the virion. Virus uptake by professional antigen-presenting cells is also facilitated by Fc-dependent interactions resulting in the presentation of viral antigens to B and T cells and amplification of the antiviral response. The binding of antibodies to infected cells can result in both cell lysis and inhibition of viral replication, viral release or viral cell–cell transmission (Dorner and Radbruch 2007).

The role of the humoral immune response during HCV infection is still poorly understood. Acute HCV patients often do not exhibit detectable neutralizing antibody titers. Their development is significantly delayed and the existing antibody response does not correlate with viral clearance (Logvinoff et al. 2004). In contrast, most chronic HCV patients possess reactive neutralizing antibodies in their serum (Bartosch et al. 2003; Logvinoff et al. 2004). These neutralizing antibodies recognize epitopes of the viral envelope, mostly located in the hypervariable region of HCV E2 protein (Bartosch et al. 2003). The fact that the present antibodies are ineffective in terminating the ongoing HCV infection can be explained by the rapid viral evolution involving a selection of viral quasispecies, which escape the reactive HCV-specific antibodies.

Interestingly, many extrahepatic manifestations of HCV infection, e.g., cryoglobulinemia, vasculitis, non-Hodgkin lymphoma and Sjögren syndrome are related to B cells (Mayo 2003). Chronic antigen stimulation by HCV was discussed as a mechanism causing HCV-associated B cell lymphoma (de Re et al. 2007). HCV proteins involved in the promotion of B cell proliferation are HCV E2 and NS3. Clonal B cell proliferation may be initiated by HCV E2/CD81 interaction (Curry et al. 2003) and by binding of HCV NS3/IgG antigen to the B cell receptor (De Re et al. 2006). HCV possibly exerts its effects on B cells by infecting them. HCV RNA was detected in B cells from HCV-infected patients but the number of RNA copies per cell seems to be rather low (Pal et al. 2006; Radkowski et al. 2005). Furthermore, while there may be B cell-specific HCV strains, cell culture-derived HCV particles could not infect peripheral blood mononuclear cells (PBMCs) including B cells (Marukian et al. 2008).

T Cell Response in Acute HCV Infection

An acute HCV infection is usually asymptomatic. Studies in experimentally HCV-infected chimpanzees demonstrate that the viral load in the serum increases exponentially during the first weeks of infection. 4–8 weeks after the initial infection, HCV-specific T cells can be detected for the first time paralleled with an increase in alanine aminotransferase levels indicating immune-associated liver injury (Cox et al. 2005; Thimme et al. 2001; Woollard et al. 2003). The rate of spontaneous HCV clearance is estimated to be 20–25 % (Gerlach et al. 2003) and is associated with a robust, sustained and multi-specific $CD4^+$ and $CD8^+$ T

cell response (Cooper et al. 1999; Lechner et al. 2000). That a robust $CD8^+$ T cell response is important for viral clearance has been shown convincingly in the chimpanzee model where an early, multi-specific, intrahepatic $CD8^+$ T cell response to HCV was detected in 2/2 chimpanzees that cleared the virus and also in 1/4 chimpanzees that became chronically infected (Cooper et al. 1999).

Depletion of $CD4^+$ T cells and reinfection of two afore immune chimpanzees with HCV resulted in a persistent low-level viremia despite a functional intrahepatic $CD8^+$ T cell response, indicating that $CD4^+$ T cells are also indispensable for sufficient HCV clearance (Grakoui et al. 2003). In humans with controlled HCV infection, the diversity of virus-specific epitopes recognized by $CD4^+$ T cells was much higher than in those who failed to control the infection (Day et al. 2002). Thus, a robust, sustained and multi-specific $CD4^+$ and $CD8^+$ T cell response seems to be required for viral clearance. Analysis of the cytokine profile in PBMCs and $CD4^+$ T cells from acutely infected HCV patients further indicated that viral clearance was largely correlated to a predominant Th1 $CD4^+$ T cell profile (production of IFN-γ and IL-2), while patients exhibiting a Th2 profile (production of IL-4 and IL-10) develop a chronic HCV infection (Tsai et al. 1997).

T Cell Response in Chronic HCV Infection

Virus-specific $CD4^+$ and $CD8^+$ T cells derived from patients with chronic HCV infection showed impaired effector function with reduced proliferative response, diminished peptide-specific cytotoxicity and decreased IFN-γ production (Gruener et al. 2001; Wedemeyer et al. 2002). The frequency of virus-specific $CD8^+$ T cells from chronically infected HCV individuals was found to be about 30-fold higher in the liver as compared to the peripheral blood (He et al. 1999). However, the majority of these T cells exhibit phenotypic characteristics consistent with an incomplete differentiation (Appay et al. 2002). The question is which mechanisms are responsible for the failure of the HCV-specific T cell response to eradicate the virus and how HCV is inducing these mechanisms. We will describe the recent achievements in answering these questions in the following part of the review.

HCV-Mediated Effects on Adaptive Immunity

Viral Escape

The virally encoded HCV polymerase lacks a proofreading function so that HCV exists in each infected host as a swarm of genetically different variants called quasispecies. Pairing with a high replication rate of about 10^{12} virions per day (Neumann et al. 1998) allows the virus to rapidly adapt to immune pressure by selecting beneficial mutations from the pre-existing quasispecies reservoir. Selection pressure is exerted by both antibodies (Farci et al. 2000; Shimizu et al. 1994) and T cells (Chang et al. 1997; Erickson et al. 2001; Frasca et al. 1999; Seifert et al. 2004; Timm et al. 2004; Tsai et al. 1998). Regarding T cells, HCV escape has been shown to affect epitope processing (Seifert et al. 2004; Timm et al. 2004), MHC binding (Chang et al. 1997) and TCR stimulation (Chang et al. 1997; Erickson et al. 2001; Frasca et al. 1999; Tsai et al. 1998). Thus, effective T cell responses target epitopes that do not allow sequence changes because of high viral fitness costs (Uebelhoer et al. 2008).

HCV-Induced T Cell Chemotaxis

To be able to control HCV infection, the infected liver produces chemokines resulting in the recruitment of HCV-specific T cells. Chemokine receptors associated with T cell homing in the liver are CCR4, CCR5 and CXCR3. While Th1 cells express mainly CCR5 and CXCR3, Th2 cells express preferentially CCR4.

Recently, it has been shown that the expression of the CCR4 ligands, CCL17 and CCL22, is enhanced in chronic hepatitis C patients (Brenndorfer et al. 2012; Riezu-Boj et al. 2011). This results in the attraction of CCR4-expressing Th2 and Treg cells thus contributing to HCV persistence.

A variety of studies have also shown that the expression of CXCR3 ligands (CXCL9, 10 and 11) and CCR5 ligands (CCL3, 4 and 5) is increased in the liver of chronically HCV-infected patients (Zeremski et al. 2007). However, although HCV-specific effector T cells may promote HCV clearance in an early phase of the infection, they cause collateral tissue damage when the infection persists. A potential survival strategy for HCV would be to decrease the number of cytotoxic T cells in the liver to both diminish antiviral immunity and extend host survival as much as possible, thus assuring its own viability. One way to achieve this is by reducing the expression of Th1-associated chemokine receptors on $CD8^+$ T cells (Lichterfeld et al. 2002). This mechanism is also supported by a gene association study showing the importance of CCR5 gene polymorphisms for HCV pathogenesis. Patients bearing the mutation CCR5-Δ32 that abrogates CCR5 expression are characterized by a higher HCV prevalence and when infected by a higher viral load (Woitas et al. 2002). In addition, an attenuation of intrahepatic T cell immunity is also achieved by the recruitment of CXCR3-expressing Treg cells.

Apoptosis of HCV-Specific T Cells

The liver is a unique organ due to the fact that it receives blood from both the systemic circulation and the intestine. Thus, the

liver is continuously exposed to food-derived antigens and to endotoxin derived from the intestinal bacteria. To avoid constant immune activation in the liver, intrahepatic immune cells exist in a state of active tolerance, meaning that in most cases, T cell stimulation in the liver leads to Fas-mediated T cell apoptosis (Huang et al. 1994). Furthermore, it has been shown that hepatocyte-activated T cells are characterized by low expression of CD25 resulting in low production of IL-2. This causes T cell death by cytokine deprivation in a Bim-dependent manner (Holz et al. 2008).

This is of particular importance in hepatitis C, where hepatocytes are regarded as the primary site of infection. Premature death of hepatocyte-activated HCV-specific T cells diminishes the antiviral T cell repertoire and hence favors T cell tolerance and HCV persistence.

HCV-Induced T Cell Exhaustion

Chronic Hepatitis C is characterized by continuous exposure of T cells to high levels of HCV antigens resulting in chronic T cell activation and more or less dysfunctional HCV-specific T cells (Gruener et al. 2001; Lechner et al. 2000). Functions like IL-2 production and proliferative capacity are lost first, followed by TNF-α production and at the end by degranulation and IFN-γ production (Gruener et al. 2001; Lechner et al. 2000; Thimme et al. 2001; Urbani et al. 2006a; Wedemeyer et al. 2002). Interestingly, the HCV core protein can inhibit T cell proliferation through interaction with the complement receptor gC1qR (Kittlesen et al. 2000; Yao et al. 2001). Since gC1qR is expressed at higher levels on $CD8^+$ than on $CD4^+$ T cells, the suppressive effects of core are stronger on $CD8^+$ T cells than on $CD4^+$ T cells (Yao et al. 2004). The question is, which factors are contributing to which extend to the development of HCV-specific T cell exhaustion and how these exhausted T cells are characterized (Fig. 3).

Deficient $CD4^+$ T Cell Help

In the setting of a chronic viral infection, $CD4^+$ T cell function is critical and plays an important role in sustaining virus-specific $CD8^+$ T cells during a chronic viral infection (Matloubian et al. 1994). Absence of functionally deficient $CD4^+$ T cells leads to T cell exhaustion and ultimately chronic infection (Kalams and Walker 1998; Ulsenheimer et al. 2003). In chronic HCV infection, $CD4^+$ T cell responses are often weak (Day et al. 2003) and characterized by reduced IL-2 production (Semmo et al. 2005) (Fig. 3).

Inhibitory Cytokine Milieu

Immunoregulatory cytokines are centrally involved in the functional inactivation of antiviral T cells. Especially IL-10 and TGF-β have been linked to T cell exhaustion. During chronic HCV infection, HCV-specific $CD8^+$ T cells producing IL-10 and TGF-β with regulatory capacity occur (Abel et al. 2006; Alatrakchi et al. 2007). Furthermore, IL-10-producing (Tr1) and TGF-β-producing (Th3) $CD4^+$ Treg cells have been described in chronic HCV patients (Cabrera et al. 2004; Ulsenheimer et al. 2003). In addition to being produced by T cells, IL-10 can also be secreted by other cell types such as macrophages, DCs, B cells and NK cells.

Persistent viral infection in mice has been shown to significantly upregulate IL-10 production by antigen-presenting cells, leading to impaired T cell responses. Blocking of the IL-10/IL-10 receptor pathway by genetic deletion or antibody treatment restored T cell function and eliminated viral infection (Brooks et al. 2006; Ejrnaes et al. 2006). IL-10 levels are also increased in patients with chronic HCV infection (Cacciarelli et al. 1996; Reiser et al. 1997). HCV-specific T cell responses in PBMCs from chronically infected patients were restored by blocking IL-10, resulting in increased IFN-γ production (Kaplan et al. 2008; Piazzolla et al. 2000). Moreover, polymorphisms of the IL-10 promotor, which are associated with increased IL-10 production, correlated with an increased susceptibility to develop chronic HCV infection (Knapp et al. 2003; Paladino et al. 2006; Persico et al. 2006).

As outlined above, IL-10 plays a crucial role for impaired DC differentiation and subsequent T cell activation in HCV-infected individuals (Auffermann-Gretzinger et al. 2001; Bain et al. 2001; Della Bella et al. 2007; Dolganiuc et al. 2003; Kanto et al. 1999). This results in suppression of $CD4^+$ T cell response (Kanto et al. 2004, 2006), an effect which could be also mimicked by the core and NS4 protein of HCV (Brady et al. 2003; Zimmermann et al. 2008). This suppression of $CD4^+$ T cell responses by HCV can be successfully reversed by an antibody blocking the IL-10 receptor (Rigopoulou et al. 2005).

Additionally to IL-10, HCV infection is also associated with a significant increase in TGF-β1 expression in both serum and liver (Blackard et al. 2006). Furthermore, polymorphisms of the TGF-β1 promotor were significantly associated with the HCV clearance rate (Kimura et al. 2006). HCV increases hepatocyte TGF-β1 expression through the generation of reactive oxygen species in a nuclear factor-κB-dependent manner, while HCV core and NS3–NS5 proteins seem to be involved (Bataller et al. 2004; Lin et al. 2010). Besides being a key factor in hepatic fibrosis development, TGF-β is also involved in the generation of inducible Tregs and maintenance of Treg function (Marie et al. 2005; Yamagiwa et al. 2001). Similar to IL-10, functional blockade of TGF-β enhances peripheral HCV-specific T cell responses (Alatrakchi et al. 2007).

These data suggest that both IL-10 and TGF-β play an important role for the development of HCV persistence (Fig. 3).

Inhibitory TCRs

Prolonged and/or high expression of multiple inhibitory receptors is a key feature of T cell exhaustion in both animal models and humans. Increased expression of PD-1 is a major mechanism by which virus-specific $CD8^+$ T cells become functionally impaired and PD-1 is up-regulated by exhausted T cells during chronic HCV infections (Golden-Mason et al. 2007; Radziewicz et al. 2007). Interaction of PD-1 with PD-L1, which is expressed by liver sinusoidal cells, Kupffer cells, hepatic stellate cells and DCs (Chen et al. 2006; Iwai et al. 2003) and is IFN-α- and IFN-γ-dependently upregulated on hepatocytes (Muhlbauer et al. 2006), induces T cell apoptosis and inhibits T cell functionality (Iwai et al. 2003; Radziewicz et al. 2007). Of note, blockade of PD-L1/PD-1 interaction resulted in the functional restoration of blood-derived HCV-specific $CD8^+$ T cell responses in chronic HCV infection (Golden-Mason et al. 2007, 2008a; Penna et al. 2007; Radziewicz et al. 2007; Urbani et al. 2006b).

Besides PD-1, many other inhibitory TCRs co-regulate T cell exhaustion. In chronic hepatitis C, HCV-specific $CD8^+$ T cells have been reported to express inhibitory receptors such as 2B4 (CD244), CD160, CTLA-4, KLRG1 or TIM-3 (Bengsch et al. 2010; Golden-Mason et al. 2009; Nakamoto et al. 2008; Schlaphoff et al. 2011) (Fig. 3). The importance of these receptors became evident, when it was shown that PD-1 blockade alone was unable to restore the function of liver-derived HCV-specific $CD8^+$ T cells (Nakamoto et al. 2008) but that additional blockade of CTLA-4 reinvigorated the antiviral T cell functions (Nakamoto et al. 2009). Additional studies are needed to better understand the interaction of these receptors and their role in the various stages of T cell exhaustion.

Induction of Tregs

Furthermore, HCV also interferes with Treg function, thereby contributing to dysregulation of the antiviral adaptive immune response (Fig. 3). Several reports indicate that the frequency of $CD4^+/CD25^+$ Tregs is increased in chronic HCV patients (Boettler et al. 2005; Cabrera et al. 2004; Rushbrook et al. 2005). It was shown that Tregs expand during the acute phase of HCV infection (Perrella et al. 2006b; Ulsenheimer et al. 2003), maintain their number during the chronic phase (Boettler et al. 2005; Cabrera et al. 2004; Sugimoto et al. 2003) and decrease their number to the level of healthy controls when the patients cure the HCV infection (Boettler et al. 2005; Sugimoto et al. 2003). Depletion of these cells resulted in an enhanced HCV-specific T cell response indicating that these cells are also involved in the suppression of HCV-specific T cell responses (Boettler et al. 2005; Cabrera et al. 2004; Rushbrook et al. 2005). HCV-specific Tregs use both direct and indirect mechanisms for their inhibitory functions. Cell-to-cell contact was shown to be indispensable for suppression by Tregs (Boettler et al. 2005; Cabrera et al. 2004). Furthermore, IL-10 and TGF-β secretion are important for HCV-specific Treg functions (Alatrakchi et al. 2007; Cabrera et al. 2004).

However, the mechanisms by which HCV might induce the recruitment of Tregs to the infected liver are not known. The recruitment of immune cells in the liver is mainly dependent on the release of specific chemokines. Thus, the recently reported increase of CCL17 and CCL22 expression found in the liver of HCV-infected patients may be of importance (Brenndorfer et al. 2012; Riezu-Boj et al. 2011). Contact of DCs with HCV-infected Huh7 cells strongly stimulates the expression of CCL17 and CCL22, which act as attractants for Tregs (Riezu-Boj et al. 2011). NS3/4A may be the HCV protein mediating the described effects since mice with liver-specific expression of NS3/4A are characterized by enhanced intrahepatic levels of CCL17 and CCL22 resulting in increased numbers of $CCR4^+$ $CD4^+$ T cells in the livers of NS3/4A-transgenic mice (Brenndorfer et al. 2012).

Immunotherapy for HCV Infection

In the last years, a substantial progress has been made in the understanding of the HCV life cycle and the mechanisms used by HCV to evade host immunity. This resulted in the discovery of various new therapy options, which may lead to the development of better immunotherapies or efficient vaccines in the future.

The current standard of care therapy for HCV-infected patients is based on pegylated IFN-α and ribavirin, which are both immunomodulatory agents. IFN-α has not only direct antiviral actions by inducing the expression of protein kinase R or 2′,5′-oligoadenylate synthetase but also effects on cellular immunity. IFN-α is known to stimulate the activation of NK cells, the maturation of DCs, the proliferation of memory T cells, the expression of MHC molecules and the promotion of Th1 cells (Maher et al. 2007). The treatment with pegylated IFN-α is thought to boost the actions of endogenous IFN-α causing a strengthening of antiviral immunity.

For ribavirin, several antiviral mechanisms have been proposed. Besides direct mechanisms resulting in the blockade of HCV replication, ribavirin is thought to increase the expression of IFN-stimulated genes (Thomas et al. 2011), modulate cytokine production by macrophages (Ning et al. 1998) and stimulate a Th1-dominated antiviral response which favors viral clearance (Hultgren et al. 1998).

HCV proteins such as NS3/4A and NS5A have not only essential functions for the viral life cycle but are also known to modulate intrahepatic immunity. NS3/4A is blocking signaling pathways in HCV-infected cells by cleaving mitochondrial antiviral signaling protein (Li et al. 2005b; Meylan et al. 2005), Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (Li et al. 2005a) and T cell protein tyrosine phosphatase (Brenndorfer et al. 2009). Furthermore, it is influencing macrophage and T cell recruitment and functionality by modulating cytokine and chemokine levels in the liver (Brenndorfer et al. 2010, 2012). NS5A was also shown to impair both the innate and adaptive hepatic immune response (Kriegs et al. 2009). Thus, future HCV therapies based on NS3/4A protease and NS5A inhibitors may exert effects beyond the viral replication by reversing HCV-mediated effects on host immunity as well.

Although the development of HCV vaccines in the last years was promising, there is still no prophylactic or therapeutic vaccine for HCV available. Vaccination experiments in chimpanzees have shown that protective immunity to both homologous and heterologous HCV strains exists (Bassett et al. 2001; Lanford et al. 2004; Weiner et al. 2001) indicating the presence of epitopes, which are conserved between genotypes. Patient studies analyzing the immune response in acute HCV infection revealed that a strong and sustained HCV-specific T cell response targeting multiple epitopes is associated with a self-limited course of infection (Rehermann 2009). Thus, a successful HCV vaccine should be able to raise these T cell responses. Since HCV NS antigens contain both highly conserved gene regions and multiple $CD4^+$ and $CD8^+$ T cell epitopes, most new HCV vaccine approaches have focused on inducing T cell responses to HCV NS antigens. By using an NS3/4A-transgenic mouse model that somewhat mimics the human infection with respect to a dysfunctional T cell response, it was shown that modulation of PD-1 and Tregs can restore T cell responsiveness (Chen et al. 2011). In the same model, it was shown that the recruitment of heterologous T cells to the site of T cell activation had the same effect (Chen et al. 2011). The immunogen itself and the administration routes have a central role in what type of T cells becomes activated (Gill et al. 2010; Lazdina et al. 2001; Nystrom et al. 2010).

Several HCV vaccine approaches based on peptides, DNA, vectors and recombinant proteins have recently reached phase I/II human clinical trials. A therapeutic peptide vaccine currently in clinical development is IC41 consisting of five synthetic peptides from the proteins core, NS3 and NS4 proteins which are conserved across the HCV genotypes 1 and 2 and are combined with the adjuvant poly-L-arginine (Firbas et al. 2010; Klade et al. 2008). A therapeutic DNA vaccine based on NS3/4A given in combination with in vivo electroporation is currently in a phase I/IIa clinical trial (Sallberg et al. 2009). Vector-based vaccines use the modified vaccinia Ankara (MVA) virus or adenovirus for gene delivery. A phase I clinical trial assessing the therapeutic vaccine TG4040 which is a MVA-based vaccine expressing NS3, NS4 and NS5B was recently completed (Habersetzer et al. 2011). In a further phase I clinical trial, healthy volunteers have been vaccinated with adenovirus vectors expressing NS3–NS5B (Barnes et al. 2012). To avoid preexisting immunity, rare serotype adenoviral vectors have been used. Interestingly, sustained HCV-specific immune responses responding to multiple HCV epitopes and HCV strains could be induced. Thus, this strategy may be suitable for both the use as prophylactic and therapeutic HCV vaccine. A further candidate for a prophylactic HCV vaccine based on a recombinant E1/E2 heterodimer adjuvanted with MF59C was also tested recently in a phase I trial (Frey et al. 2010).

In addition, cell-based immunotherapies using HCV antigen-presenting DCs or HCV-specific T cells have been used as vaccine approaches. In a phase I trial, DCs were harvested and then loaded and activated with HCV-specific cytotoxic T cell epitopes before they were reinjected in HCV-infected patients (Gowans et al. 2010). In a preclinical study, mice have been vaccinated against HCV with DCs transduced with an adenovirus encoding NS3 protein (Echeverria et al. 2011). Additionally, HCV TCR-transduced T cells, which have only been tested in preclinical studies till now, may be promising for the treatment of patients with chronic HCV infection (Pasetto et al. 2012; Zhang et al. 2010).

Thus, several promising vaccine trials have been completed recently and are also planned for the near future. However, since the described therapeutic HCV vaccine studies have been characterized by an induction of strong T cell responses but only a weak and transient drop of the viral load, therapeutic vaccines may be used in the next years in combination with the standard HCV therapy or future DAA regimens rather than isolated.

Conclusions

An effective cellular immune response is critical for HCV eradication during the acute phase of the infection. However, HCV has developed a big variety of mechanisms interfering with antiviral immunity so that in the majority of patients infected with HCV a chronic infection is established. In the chronic phase of the infection, persistent activation of cellular immunity without successful elimination of the virus results in chronic inflammation leading to liver injury and liver cirrhosis. HCV is preventing a higher degree of liver damage by simultaneously attenuating innate and adaptive

immune responses allowing coexistence of both virus and host. Blocking the HCV-mediated impairment of cellular immunity paralleled by an effective stimulation of the HCV-specific immune response is necessary to be able to clear the virus. Since in the last years fantastic efforts have been made in the characterization of the HCV-mediated mechanisms responsible for HCV persistence, we hope that these efforts will make it possible to develop effective immunotherapies or therapeutic vaccines against HCV.

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Conflict of interest None to declare.

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