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REVIEW

Virus-Specific Cellular Response in Hepatitis C Virus Infection

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Abstract Studies performed on chimpanzees and humans have revealed that strong, multispecific and sustained $CD4^+$ and $CD8^+$ T cell immune responses is a major determinant of hepatitis C virus (HCV) clearance. However, spontaneous elimination of the virus occurs in minority of infected individuals and cellular response directed against HCV antigens is not persistent in individuals with chronic infection. This review presents characteristics of the HCV-specific T cell response in patients with different clinical course of infection, including acute and chronic infection, persons who spontaneously eliminated HCV and non-infected subjects exposed to HCV. Detection of HCV-specific response, especially in non-infected subjects exposed to HCV, may be indicative of HCV prevalence in population and rate of spontaneous viral clearance. Understanding the mechanisms and role of HCV-specific cellular immune response would contribute to better understanding of HCV epidemiology, immunopathogenesis and may help to design an effective vaccine.

Keywords HCV · Virus-specific cellular response · $CD4^+$ · $CD8^+$ T cells

Abbreviations

Bcl-2	B-cell lymphoma 2
DCs	Dendritic cells
HCV	Hepatitis C virus
HCV	RNA hepatitis C viral genetic material
HLA-DR	Human leukocyte antigen D related
PWIDs	People who inject drugs
IFN-γ	Interferon γ
IL-2	Interleukin 2
Th1	T helper cells
TNF	Tumor necrosis factor

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Introduction

In the majority of cases (60–85 %) hepatitis C virus (HCV) infection is primarily chronic since the virus efficiently evades adaptive immunity (Maheshwari et al. 2008; Riviere et al. 2012; Sarobe et al. 2002; Strickland et al. 2008) and the late consequences of infection are liver cirrhosis and hepatocellular carcinoma (Neumann-Haefelin et al. 2005; Tatsumi et al. 2011). The main factor determining elimination of HCV is effective immune response (Zhang et al. 2009).

Mechanisms of acquired immune response against HCV antigens include both humoral and cellular components; however, cell-mediated response is believed to play a principal antiviral role (Neumann-Haefelin et al. 2005). The strength and breadth in patients who spontaneously eliminate HCV are the crucial determinants of virus eradication. Only in approximately 20 % of infected individuals who spontaneously clear the virus broad HCV-specific $CD4^+$ and $CD8^+$ T cell responses are observed (Cox et al. 2005; Kamal et al. 2002; Neumann-Haefelin et al. 2005).

In contrast, humoral response was proved to be less important in the elimination of HCV (Post et al. 2004; Takaki et al. 2000). The serological window period, or the

time elapsed from the onset of infection to produce detectable titers of anti-HCV antibodies ranges from 6 weeks to 9 months, although it usually does not exceed 12 weeks (Arrojo et al. 2003; Lauer and Walker 2001; Thomson et al. 2009). It was noticed that in some patients eliminating HCV, humoral immunity can be even absent, despite the detection of a cellular response to number of HCV antigens (Al-Sherbiny et al. 2005; Post et al. 2004; Scognamiglio et al. 1999). In patients with hypogammaglobulinemia, lacking a vigorous humoral response, similar incidence of spontaneous virus elimination as in the general population is observed. Furthermore, no relationship was found between the level of anti-HCV and the elimination of the pathogen (Bjoro et al. 1994; Chapel et al. 2001; Razvi et al. 2001). Sequencing of the hypervariable region of HCV (HVR1) from peripheral blood of infected patients suggests that the activity of anti-HCV antibodies may play a role in the elimination of infecting (baseline) variants of the virus but not the emerging viral quasispecies (Scarselli et al. 1995).

Methods of Cellular Response Analysis

Evaluation of HCV-specific cellular response involves in vitro stimulation of immune cells isolated from peripheral blood or liver tissue with HCV antigens, which are structural (Core) and non-structural (NS3, NS4 and NS5) proteins. After stimulation HCV-specific cellular response is assessed employing proliferation or cytotoxicity assay (Badr et al. 2008; Cramp et al. 1999; Freeman et al. 2003; Gruner et al. 2000), intracellular staining of cytokines secreted by Th1 cells (Bolacchi et al. 2006), enzyme-linked immunospot assay; (Gruner et al. 2000; Takaki et al. 2000; Zeremski et al. 2009) or flow cytometry (Bolacchi et al. 2006; Day et al. 2002; Post et al. 2004; Rosen et al. 2002). The latter enables simultaneous evaluation of activation status e.g., markers of activation or cytokine secretion in distinct cell populations present in a single sample (Lafarge et al. 2007).

Chimpanzee Model for HCV Infection

Since the early stages of HCV infection are usually asymptomatic, investigation of development of immune response in humans is difficult. Thus, numerous studies were conducted on chimpanzees which are the only available animal model of HCV infection (Bassett et al. 2001; Elliot et al. 2006; Farci et al. 1992; Grakoui et al. 2003; Lai et al. 1994; Major et al. 2002; Prince et al. 1992; Shoukry et al. 2003). Although, they provided some light into the mechanisms of HCV infection, it should be noted that most of the research has been performed on small number of animals: typically one or two (Grakoui et al. 2003; Shoukry et al. 2003). Another limitation of this model is that chimpanzee experiences milder course of infection and much more frequent spontaneous HCV elimination than human (Elliot et al. 2006).

Studies on chimpanzees revealed a clear relationship between the spontaneous elimination of HCV and T-cell response (Grakoui et al. 2003; Shoukry et al. 2003). The elimination of the virus during secondary exposure in animals, which cleared primary infection, can occur even in the absence of anti-HCV antibodies (Major et al. 2002) whereas memory $CD4^+$ (Shoukry et al. 2003) and $CD8^+$ (Grakoui et al. 2003) T cells play a key role. It is known that presence of sustained $CD4^+$ T-cell response during first and subsequent infection is required for priming activation and maintenance of $CD8^+$ T cells in chimpanzees, humans and rodents (Grakoui et al. 2003; Shedlock and Shen 2003; Sun et al. 2004). The depletion of $CD4^+$ T cells in vivo enabled to determine their impact on the course of HCV infection. It resulted in reduced frequency of antigen-specific $CD8^+$ T cells and their activity and more frequent emergence of mutations within HCV epitopes recognized by these cells (Badr et al. 2008; Zhang et al. 2009).

The secondary infection in chimpanzees is characterized by early cytotoxic activity of $CD8^+$ cells, intrahepatic antiviral cytokine synthesis [interferon (IFN)-γ and tumor necrosis factor (TNF)]; (Major et al. 2002) and increase in the number of memory $CD4^+$ and $CD8^+$ T cells in peripheral blood (Grakoui et al. 2003; Shoukry et al. 2003). Due to the effects of HCV-specific cells active in secondary infection with the same or different HCV genotype the observed viral load is usually lower and virus is eliminated more rapidly (Bassett et al. 2001; Elliot et al. 2006; Farci et al. 1992; Major et al. 2002; Prince et al. 1992; Weiner et al. 2001).

The effect of $CD8^+$ T cells depletion was studied in chimpanzees which spontaneously eliminated HCV twice, and then were re-infected. The decrease in activity of $CD8^+$ T cells in the liver was associated with longer viral load persistence (6 weeks) than during the second infection (2 weeks), although shorter than during the first challenge (12–20 weeks); (Shoukry et al. 2003).

Cellular Response Against HCV in Human

Similar to animal models, cellular immune response plays a principal role in the clinical course of HCV infection and virus elimination in humans. Determination of the strength and range of recognized viral epitopes in individuals who spontaneously clear the virus seems to be crucial in understanding the mechanisms of eradication of infection.

As mentioned, only minority HCV infected subjects experience spontaneous elimination of the virus, which is the result of the activity of intrahepatic and peripheral blood $CD4^+$ and $CD8^+$ T cells, specific to structural and non-structural HCV antigens (Kamal et al. 2002; Neumann-Haefelin et al. 2005; Zhang et al. 2009).

Acute Infection

Cellular response during the acute phase of HCV infection is critical for rapid viral clearance. Within six months of the onset of symptoms, in the majority of individuals who ultimately spontaneously eliminate the virus, cellular $CD4^+$ and $CD8^+$ T-cell responses can be detectable to structural and non-structural HCV antigens (Aberle et al. 2006; MacDonald et al. 2002; Spada et al. 2004; Ulsenheimer et al. 2003). It is believed that this response control virus replication (Aberle et al. 2006; Day et al. 2002; Post et al. 2004) and multispecific T-cell response during the early phase of acute HCV infection are associated with disease resolution and spontaneous clearance (Spada et al. 2004).

The most important factors contributing to spontaneous elimination of HCV are both the number and activity of antigen-specific $CD8^+$ T cells (Gruner et al. 2000). Their antiviral function is based on cytotoxicity against cells infected with HCV and the secretion of pro-inflammatory and virus-inhibitory cytokines in particular IFN-γ (Elliot et al. 2006; Neumann-Haefelin et al. 2007). During the early acute phase, in subjects eliminating the virus, secretory function of these cells is temporarily inhibited, followed by its restoration (Lechner et al. 2000; Thimme et al. 2001). Simultaneously, HCV-specific $CD4^+$ T cells provide help for $CD8^+$ T cells through the secretion of cytokines (interleukin (IL)-6, IFN-γ, TNF) and activation of antigen-presenting cells (Semmo and Klenerman 2007). It was observed that specific $CD4^+$ T cells show high proliferative capacity in the acute phase of infection both of humans (Sarobe et al. 2002) and chimpanzees (Semmo and Klenerman 2007; Thimme et al. 2001) facilitating elimination of the virus.

In individuals with acute HCV infection, a positive correlation was found between strong $CD4^+$ T-cell-mediated response and spontaneous elimination of the virus (Neumann-Haefelin et al. 2007; Urbani et al. 2006). In those out of them who finally develop a chronic form of infection a specific cellular response is detectable but it is characterized by low intensity and is directed against few viral epitopes. Moreover, HCV-specific T cells are characterized by a $CD127^{lo}$ $Bcl\text{-}2^{lo}$ phenotype, having limited activity due to diminished proliferation rate and cytokine synthesis, and are not detectable in the peripheral blood (Badr et al. 2008). The lack of antiviral cellular response during the first months of infection indicates the persistence of the virus and development of the chronic infection (Aberle et al. 2006).

Chronic Infection

Different studies of HCV-specific cellular response among persistently infected individuals can be detectable in a surprising broad range from 12.5 to 69.0 % of (Chang et al. 2001; Day et al. 2002; Riviere et al. 2012; Rosen et al. 2002; Zhang et al. 2009). Majority of studies report that the reactivity of $CD4^+$ (Kamal et al. 2002) and $CD8^+$ (Barnes et al. 2002; Freeman et al. 2005; Pilli et al. 2007; Tatsumi et al. 2011) T cells in these patients occur less frequent, is less vigorous and directed against fewer HCV epitopes in comparison to patients with acute phase of infection (Rosen et al. 2002; Schulze zur Wiesch et al. 2005; Tatsumi et al. 2011) and spontaneously eliminating the virus (Aberle et al. 2006, 2007).

$CD4^+$ Th Cell Response

The percentage of HCV-specific $CD4^+$ T cells in the peripheral blood from individuals with chronic infection is usually very low or even not detectable (0–0.001 %); (Lucas et al. 2007; Zhang et al. 2009). Some studies showed that the proportion of these cells may reach 1.1 %, but the results were obtained after several days of stimulation with HCV antigens in vitro (Day et al. 2002). Antigen-specific $CD4^+$ T cells are characterized usually by impaired proliferation and cytokine secretion, e.g., IFN-γ and IL-2 (Semmo et al. 2007) and respond to limited number of HCV epitopes (Day et al. 2002; Semmo and Klenerman 2007).

In chronic HCV carriers the HCV-specific $CD4^+$ T cells reactive to core and/or non-structural antigens (NS3, NS4, NS5) are more frequently detectable in the liver tissue than in the peripheral blood (Schirren et al. 2000). This suggests that the extent of specific responses of these cells may be compartmentalized to liver tissue which is the principal site of inflammation (Day et al. 2002; Minutello et al. 1993; Rosen et al. 2002).

$CD8^+$ T-Cytotoxic-Cell Response

It is known that the activity of virus-specific $CD8^+$ T cells in the course of chronic HCV infection is usually disturbed (Elliot et al. 2006; Neumann-Haefelin et al. 2007; Zhang et al. 2009). Surprisingly, some researchers reported contrary results, showing that these cells are widely distributed in the peripheral blood and liver tissue, and are stimulated in response to a greater number of different HCV antigens compared to those who spontaneously eliminate HCV

(Cooper et al. 1999; Scognamiglio et al. 1999; Wedemeyer et al. 2002).

HCV-specific T cells range 0–1.2 % of all $CD8^+$ T cells in peripheral blood of chronically infected subjects (He et al. 1999; Wedemeyer et al. 2002; Zhang et al. 2009). Usually they exhibit abnormal cytotoxic activity, cytokine secretion and proliferation after specific antigen stimulation in vitro (Badr et al. 2008; Boettler et al. 2005; Harcourt et al. 2001; Minton et al. 1998; Thursz et al. 1999; Wedemeyer et al. 2002). It has been shown that the HCV-specific $CD8^+$ T cells display low levels of CD27 and CD28 and high HLA-DR expression indicating mature phenotype and activation of these cells (Wedemeyer et al. 2002). So far it is not clear whether impaired activity of $CD8^+$ T cells during the chronic infection is the cause or rather the consequence of chronic infection with long-term and massive viral antigen stimulation (Badr et al. 2008). It was noticed that in chronically HCV infected patients IFN-free therapy result in restoration of HCV-specific $CD8^+$ T cell (Martin et al. 2014).

Another type of chronic HCV infection is occult infection identified in anti-HCV and HCV RNA serum negative individuals. Occult HCV infection is usually determined by the HCV RNA presence in liver tissue, abnormal liver function and histological damage (Carreno 2006; Carreno et al. 2012; Castillo et al. 2004; Quiroga et al. 2006). The term "occult HCV" infection is also used for individuals who cleared HCV infection but display HCV RNA in liver and anti-HCV antibodies in blood serum (Carreno 2006). In these patients, virus-specific effector and memory $CD4^+$ and $CD8^+$ T cells are detectable and may persist in the peripheral blood (Al-Sherbiny et al. 2005; Kamal et al. 2004; Quiroga et al. 2006; Scognamiglio et al. 1999). Therefore, HCV-specific cellular immune response may be indicative as a marker of previous exposure and recovery from HCV or of ongoing occult HCV infection (Quiroga et al. 2006).

The Possible Mechanisms of Development of a Chronic Infection

Results of multiple studies suggest several reasons for inefficient cellular response to HCV antigens, inability to eliminate HCV and the development of chronic infection. One of principal mechanism may be direct infection of dendritic cells (DCs) by the virus and consequently impaired processing and presentation of viral antigens to T cells (Bain et al. 2001; Sarobe et al. 2002; Zhang et al. 2009). Thus, impaired antigen presentation by DCs prevents primary $CD4^+$ and $CD8^+$ T cells activation and cause T-cell failure or exhaustion which are important factors determining viral persistence (Bain et al. 2001; Erickson et al. 2001; Kantzanou et al. 2003). Immune exhaustion is defined as weak antigen-specific T-cell response manifested as impairment of antiviral effector functions of these cells. It includes decline in effector cytokines synthesis, elimination of infected cells and decrease in proliferate potential after antigen exposure in vitro (Penna et al. 2007). Immune exhaustion is mediated by continuous and massive antigen stimulation which progress along with time of infection (Yi et al. 2010).

The essential mechanisms of T-cell dysfunction or anergy of HCV-specific T cells are: (1) abnormal maturation of $CD8^+$ T cells. Consequently, HCV-specific $CD8^+$ T cells are in immature phenotype, $CD28^+$ and/or $CD27^+$, are less cytotoxic and display impaired synthesis of IFN-γ (Appay et al. 2002; Wedemeyer et al. 2002), (2) impaired activity of regulatory T cells which modulate the activity of $CD4^+$ and $CD8^+$ T cells by secreting IL-10 and transforming growth factor-β (Boettler et al. 2005; Groux et al. 1996, 1997; Rushbrook et al. 2005), (3) immunosuppression by viral proteins and genetic material responsible for reduced activity of DCs, T and NK cells (Waggoner et al. 2007), and (4) high expression level of programmed death 1 receptor on $CD8^+$ T cells resulting in the exhaustion of specific memory cells (Neumann-Haefelin et al. 2005; Radziewicz et al. 2007; Semmo and Klenerman 2007; Zhang et al. 2009).

Generally accepted principal mechanism affecting the function of HCV-specific cells is mutations in the viral genome, in particular during the early stage of infection (Chang et al. 2001; Cox et al. 2005; Kuntzen et al. 2007; Neumann-Haefelin et al. 2005; Tester et al. 2005; Timm et al. 2004). High rate of HCV replication and no proof-reading activity of RNA-dependent RNA polymerase facilitate the emergence of escape mutations in genes encoding epitopes recognized by $CD4^+$ and $CD8^+$ T cells. Consequently, immune cells are not able to recognize and control newly produced viral escape mutation (Cox et al. 2005; Tester et al. 2005; Timm et al. 2004). Reduced viral fitness can limit the variability of HCV within immunological epitopes resulting in frequency reduction of viral escape mutations (Söderholm et al. 2006).

Finally, human leukocyte antigen (HLA) class I and II play a role in the pathogenesis of HCV infection by presenting antigens to T cells. HLA alleles may have an impact on the T-cell response outcome. Polymorphism within the HLA binding region affects the presentation of viral antigens, strength of the cellular response and range of HCV epitopes recognized (Neumann-Haefelin et al. 2007). For example, A*08 and B*18 alleles are more frequently found in chronic HCV infection (McKiernan et al. 2004), whereas B*27 allele is associated with viral clearance (Neumann-Haefelin et al. 2006).

Spontaneous Elimination of HCV

The vast majority of individuals who spontaneously eliminate HCV display a strong, broad and sustained HCV-specific $CD4^+$ T cell response (Chang et al. 2001; Cramp et al. 1999; Rosen et al. 2002; Welsh and Selin 2002), whereas HCV-specific $CD8^+$ T cell response is noticed in wide range 19–85 % recovered individuals depending on assay type (Chang et al. 2001; Lauer et al. 2004). Virus elimination occurs usually within 12–24 weeks after the onset of infection (Badr et al. 2008; Page et al. 2009). In vitro studies in these patients show that non-structural antigens (NS2, NS3, NS4, NS5) are more immunogenic than HCV core antigen (Aberle et al. 2006; Schulze zur Wiesch et al. 2005). HCV clearance is mediated by vigorous T-cell response in the liver with cytotoxic and non-cytotoxic effector functions (Neumann-Haefelin et al. 2005).

The presence of strong HCV-specific CD4+ T-cell responses to a number of viral epitopes is essential to stimulate $CD8^+$ T cells (Gruner et al. 2000; Lauer et al. 2004; Neumann-Haefelin et al. 2007). In patients eliminating infection, the percentage of specifically primed $CD4^+$ T cells after in vitro stimulation with HCV antigens ranges 0.01–1.1 % in peripheral blood (Gruner et al. 2000; Lucas et al. 2007; Wedemeyer et al. 2002), and may reach 26 % if based on determination of percentage of IFN-γ secreting cells after several days of stimulation with HCV antigens (Day et al. 2002; Wedemeyer et al. 2002). The reactivity of these cells is detectable even several years after the virus elimination (Day et al. 2002).

In vitro studies performed on $CD8^+$ T cells have shown that in subjects eliminating the virus, HCV-specific T cells represent 0–0.2 % of this population in peripheral blood (Gruner et al. 2000; Wedemeyer et al. 2002). Virus clearance is associated with early appearance and gradual expansion of multifunctional $CD8^+$ T cells (IFN-γ^+, IL-2^+, CD107a^+ phenotype), with increased expression of the memory marker CD127 and anti-apoptotic protein Bcl-2. They exhibit T_{CM} (rapid proliferation, high levels of IL-2) and T_{EM} (high IFN-γ secretion and cytotoxic potential) characteristics. Even several years after spontaneous elimination of HCV, memory $CD8^+$ cells can be detectable in peripheral blood (phenotype $CD45RO^+$), which exhibit high expression of CD27 and CD28 and low expression of HLA-DR. This population indicates the clearance of the previous infection and the lack of current exposure to the viral antigens (Badr et al. 2008).

In contrast to the specific memory cells in patients with chronic infection, these cells, do not exhibit ex vivo cytotoxicity, however, after in vitro stimulation with HCV antigens, they display immediate effector functions: proliferation, cytotoxicity and secretion of IFN-γ; (Wedemeyer et al. 2002). Spontaneous elimination of HCV may be mediated by the cytotoxic effect of $CD8^+$ cells leading to the destruction of infected cells or the secretion of IFN-γ and TNF fallowed by inhibition of viral replication (Guidotti and Chisari 2001; Neumann-Haefelin et al. 2005).

People who inject drugs (PWIDs) frequently exposed to HCV, who had spontaneously eliminated HCV, a lower risk of re-infection and development of chronic infection was observed than in individuals without previous exposure to HCV. Furthermore, secondary infection in PWIDs was characterized by lower and rapid declining viral load than during the primary infection (Badr et al. 2008; Grebely et al. 2006; Mehta et al. 2002; Page et al. 2009). Thus, an effective immune response during the primary infection seems to have some protective effect against the development of chronic infection during the subsequent exposure with the virus (Houghton and Abrignani 2005). In individuals who have spontaneously eliminated the virus, HCV-specific memory cells are detectable even 20 years after the virus eradication (Takaki et al. 2000) and its persistence is caused probably by cytokine-induced proliferation (Welsh and Selin 2002). Alternatively, the reason for the detection of HCV-specific cells may be exposure of the immune system to cross-reactive antigens which non-specifically stimulate memory cells (Al-Sherbiny et al. 2005; Gruner et al. 2000; Prlic et al. 2002). For example, there is clear sequence homology between HCV NS5B protein with hepatitis B virus envelope protein (Kennedy et al. 2006). Also, the occurrence of cross-reactivity between the non-structural NS3 antigen and influenza virus neuraminidase was demonstrated (Kennedy et al. 2006). It is also possible that the maintenance of memory T cells is caused by very low level but continuous viral replication (Shoukry et al. 2003).

Non-Infected Individuals at Risk of Exposure to HCV

Specific cellular response to HCV can be present in some individuals exposed to the virus, with no evidence of infection: anti-HCV and HCV RNA not detectable in serum and no liver damage (Hashem et al. 2011; Kamal et al. 2004; Scognamiglio et al. 1999). This type of response may be present in PWIDs, medical staff, family members, sexual partners of infected persons with frequency ranging 0–36 % (Al-Sherbiny et al. 2005; Hashem et al. 2011; Riviere et al. 2012; Scognamiglio et al. 1999). In particular, it is prevalent in more than 60 % of healthy children born to mothers infected with the virus (Della Bella et al. 2005; Hashem et al. 2011). The presence of

Table 1 Characteristics of HCV-specific T-cell immune response in respective populations

Population	Prevalence [%] (n = number of patients)	Peripheral blood cell population	HCV antigens (amino acid sequence) [genotype]	Method	References
Individuals with chronic HCV infection	16 ($n = 31$)	$CD4^+$ T cells	C (1-115), NS3 (1007-1534), NS4 (1616-1862) [1a]	Proliferation assai	(Cramp et al. 1999)
	16 ($n = 14$)	$CD4^+$ T cells	C (2-120), NS3 (1192-1457), NS4 (1569-1931), NS5 (2054-2995) [1a]	Proliferation assai	(Chang et al. 2001)
	47 ($n = 8$)	$CD8^+$ T cells		Cytotoxicity assai	
	33–40 ($n = 20$)	T cells	37 peptides covering C (1-75, 1-13, 66-135, 14-25, 126-191, 26-37) region [1a]	ELISpot	(Riviere et al. 2012)
	12.5–18 ($n = 17$)		37 peptides covering NS3 (1027-1657) region [1b]	Proliferation assay	
	45 ($n = 42$)	$CD4^+$ T cells	Core (2-120), NS3 (1192-1457), NS4 (1569-1931), NS5 (2054-2995) [1]	Proliferation assai	(Kamal et al. 2002)
	52 ($n = 23$)	$CD4^+$ T cells	C (39-63), E1 (238-262), NS3 (1384-1401, 1406-1415) [1b]	Proliferation assai	(Della Bella et al. 2005)
	52 ($n = 48$)	$CD4^+$, $CD8^+$ T cells	171 peptides covering NS3, NS4 and NS5A region [1b]	ELISpot	(Zhang et al. 2009)
	69 ($n = 16$)	$CD4^+$ T cells	C (1-115), NS3 (1007-1534), NS4 (1616-1862) [1a]	ELISpot	(Rosen et al. 2002)
Individuals spontaneously eliminating HCV	64 ($n = 14$)	$CD4^+$ T cells	C (2-120), NS3 (1192-1457), NS4 (1569-1931), NS5 (2054-2995) [1a]	Proliferation assay	(Chang et al. 2001)
	19 ($n = 8$)	$CD8^+$ T cells		Cytotoxicity assai	
	66 ($n = 35$)	$CD4^+$ T cells	C (1-115), NS3 (1007-1534), NS4 (1616-1862) [1a]	Proliferation assai	(Cramp et al. 1999)
	100 ($n = 6$)	$CD4^+$ T cells	C (1-115), NS3 (1007-1534), NS4 (1616-1862) [1a]	ELISpot	(Rosen et al. 2002)
	85 ($n = 20$)	$CD8^+$ T cells	Entire HCV polypeptide [1a]	ELISpot	(Lauer et al. 2004)
Individuals at risk of HCV infection	9–30 ($n = 20$)	T cells	37 peptides covering C region (1-75, 1-13, 66-135, 14-25, 126-191, 26-37) [1a]	ELISpot	(Riviere et al. 2012)
	0 ($n = 17$)		37 peptides covering NS3 region (1027-1657) [1b]	proliferation assay	
	18 ($n = 71$)	T cells	216 peptides covering C and NS3-NS5B regions	ELISpot	(Al-Sherbiny et al. 2005)
	20 ($n = 20$)	T cells	C, NS3, NS4, NS3-NS4, NS5	proliferation assay	(Bronowicki et al. 1997)
	24 ($n = 29$)	$CD8^+$ T cells	Core (35, 132), E2 (726, 728), NS3 (1131), NS4 (1590, 1666, 1769, 1851, 1920), NS5 (2592)	cytotoxic assay	(Scognamiglio et al. 1999)
	36 ($n = 42$)	$CD4^+$ T cells	C (2-120), NS3-NS4 (1569-1931), NS5 (2054-2995) [1a]	ELISpot	(Hashem et al. 2011)
	64 ($n = 25$)	$CD4^+$ T cells	C (39-63), E1 (238-262), NS3 (1384-1401, 140-1415)	proliferation assay	(Della Bella et al. 2005)

ELISpot enzyme-linked immunospot assay

HCV-specific cellular response may be protective in these children (Della Bella et al. 2005), because most of them (96.5 %) do not develop HCV infection during pregnancy or delivery (Syriopoulou et al. 2005). Probably, the activity of $CD4^+$ T cells in fetus is induced by contact with the virus in the presence of maternal anti-HCV antibodies (Della Bella et al. 2005).

It is not clear whether the cellular response in exposed individuals is more frequent after stimulation with HCV core antigen (Della Bella et al. 2005; Riviere et al. 2012), or with non-structural antigens, in particular NS5 (Hashem et al. 2011). Surprisingly, the virus-specific cellular response in non-infected individuals exposed to HCV is more frequent, stronger and directed against greater

Table 2 Frequency of HCV-specific $CD4^+$ and $CD8^+$ T cells in humans

Population	Percentage of HCV-specific cells	T cells population	Method	References
Individuals with chronic HCV infection	0–0.001	peripheral blood $CD4^+$T cells	MHC II tetramer staining	(Zhang et al. 2009)
	0–1.2	peripheral blood $CD8^+$T cells	MHC I tetramer staining	(Wedemeyer et al. 2002)
	0.01–0.2	peripheral blood $CD8^+$T cells	MHC I tetramer staining	(He et al. 1999)
	1–2	hepatic tissue $CD8^+$T cells		
Individuals spontaneously eliminating HCV	0.01–1.1	peripheral blood $CD4^+$T cells	MHC II tetramer staining	(Zhang et al. 2009)
	0–0.2	peripheral blood $CD8^+$T cells	MHC I tetramer staining	(Wedemeyer et al. 2002)
	0–0.2	peripheral blood $CD8^+$T cells	ELISpot	(Gruner et al. 2000)

number of the viral epitopes if compared to chronically infected subjects experiencing massive antigen stimulation (Della Bella et al. 2005; Hashem et al. 2011).

It is suggested that the presence of cellular response in individuals exposed to HCV indicates the persistence of the immunological memory after asymptomatic infection followed by spontaneous viral clearance (Al-Sherbiny et al. 2005; Hashem et al. 2011; Scognamiglio et al. 1999) or reflect the asymptomatic or cryptogenic (occult) HCV infection. Confirmation of the latter clinical condition is difficult because there is no indication for liver biopsy in these groups of patients (Castillo et al. 2004; Quiroga et al. 2006).

Anti-HCV humoral response is usually not present after non-infective exposure to HCV, despite the detection of $CD4^+$ and $CD8^+$ T-cell reactivity to HCV antigens. The seronegative status is probably due to the low infective dose of the virus, insufficient to stimulate B cells for the production of anti-HCV antibodies or early destruction of antigen-specific B cells which express viral products (Koziel et al. 1997; Scognamiglio et al. 1999).

Conclusion

The research on mechanisms of specific immunological response to HCV antigens is crucial for better understanding of epidemiology and pathogenesis of infection, as well as antiviral treatment efficiency. Although vigorous and multispecific $CD4^+$ and $CD8^+$ T cell responses positively correlate with spontaneous or treatment- induced virus clearance, some important questions remain unclear. They include the causes of weak HCV-specific immune activation in chronically infected patients, exhaustion of the immune system, occult infection and viral antigen variability. The latter is a particular challenge to develop an effective anti-HCV vaccine. Finally, methodologic issues may explain differences and inconsistencies in the presented results of HCV-specific immune response.

Tables 1 and 2 present the prevalence and characteristics of HCV-specific cellular immune response. Discrepancies in the results presented can be due to differences in sensitivity of methods used, sample size and antigens used for stimulation of cells in vitro.

Compliance with ethical standards

Conflict of interest All authors of this article declare that they do not have any competing interests to the presented work.

References

Aberle JH, Formann E, Steindl-Munda P et al (2006) Prospective study of viral clearance and CD4(+) T-cell response in acute hepatitis C primary infection and reinfection. J Clin Virol 36:24–31

Aberle JH, Perstinger G, Weseslindtner L et al (2007) CD4+ T cell responses in patients with chronic hepatitis C undergoing peginterferon/ribavirin therapy correlate with faster, but not sustained, viral clearance. J Infect Dis 195:1315–1319

Al-Sherbiny M, Osman A, Mohamed N et al (2005) Exposure to hepatitis C virus induces cellular immune responses without detectable viremia or seroconversion. Am J Trop Med Hyg 73:44–49

Appay V, Dunbar PR, Callan M et al (2002) Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. Nat Med 8:379–385

Arrojo IP, Pareja MO, Orta MD et al (2003) Detection of a healthy carrier of HCV with no evidence of antibodies for over four years. Transfusion 43:953–957

Badr G, Bédard N, Abdel-Hakeem MS et al (2008) Early interferon therapy for hepatitis C virus infection rescues polyfunctional, long-lived CD8+ memory T cells. J Virol 82:10017–10031

Bain C, Fatmi A, Zoulim F et al (2001) Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. Gastroenterology 120:512–524

Barnes E, Harcourt G, Brown D et al (2002) The dynamics of T-lymphocyte responses during combination therapy for chronic hepatitis C virus infection. Hepatology 36:743–754

Bassett SE, Guerra B, Brasky K et al (2001) Protective immune response to hepatitis C virus in chimpanzees rechallenged following clearance of primary infection. Hepatology 33:1479–1487

Bjoro K, Froland SS, Yun Z et al (1994) Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. N Engl J Med 331:1607–1611

Boettler T, Spangenberg HC, Neumann-Haefelin C et al (2005) T cells with a CD4+ CD25+ regulatory phenotype suppress

in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. J Virol 79:7860–7867

Bolacchi F, Sinistro A, Ciaprini C et al (2006) Increased hepatitis C virus (HCV)-specific CD4+ CD25+ regulatory T lymphocytes and reduced HCV-specific CD4+ T cell response in HCV-infected patients with normal versus abnormal alanine aminotransferase levels. Clin Exp Immunol 144:188–196

Bronowicki JP, Vetter D, Uhl G et al (1997) Lymphocyte reactivity to hepatitis C virus (HCV) antigens shows evidence for exposure to HCV in HCV-seronegative spouses of HCV-infected patients. J Infect Dis 176:518–522

Carreno V (2006) Occult hepatitis C virus infection: a new form of hepatitis C. World J Gastroenterol 12:6922–6925

Carreno V, Bartolome J, Castillo I et al (2012) New perspectives in occult hepatitis C virus infection. World J Gastroenterol 18:2887–2894

Castillo I, Pardo M, Bartolomé J et al (2004) Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. J Infect Dis 189:7–14

Chang KM, Thimme R, Melpolder JJ et al (2001) Differential CD4(+) and CD8(+) T-cell responsiveness in hepatitis C virus infection. Hepatology 33:267–276

Chapel HM, Christie JM, Peach V et al (2001) Five-year follow-up of patients with primary antibody deficiencies following an outbreak of acute hepatitis C. Clin Immunol 99:320–324

Cooper Erickson AL, Adams EJ et al (1999) Analysis of a successful immune response against hepatitis C virus. Immunity 10:439–449

Cox AL, Mosbruger T, Mao Q et al (2005) Cellular immune selection with hepatitis C virus persistence in humans. J Exp Med 201:1741–1752

Cramp ME, Carucci P, Rossol S et al (1999) Hepatitis C virus (HCV) specific immune responses in anti-HCV positive patients without hepatitis C viraemia. Gut 44:424–429

Day CL, Lauer GM, Robbins GK et al (2002) Broad specificity of virus-specific CD4+ T-helper-cell responses in resolved hepatitis C virus infection. J Virol 76:12584–12595

Della Bella S, Riva A, Tanzi E et al (2005) Hepatitis C virus-specific reactivity of CD4+ -lymphocytes in children born from HCV-infected women. J Hepatol 43:394–402

Elliot LN, Lloyd AR, Ziegler JB et al (2006) Protective immunity against hepatitis C virus infection. Immunol Cell Biol 84:239–249

Erickson AL, Kimura Y, Igarashi S et al (2001) The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. Immunity 15:883–895

Farci P, Alter HJ, Govindarajan S et al (1992) Lack of protective immunity against reinfection with hepatitis C virus. Science 258:135–140

Freeman AJ, Pan Y, Harvey CE et al (2003) The presence of an intrahepatic cytotoxic T lymphocyte response is associated with low viral load in patients with chronic hepatitis C virus infection. J Hepatol 38:349–356

Freeman AJ, Marinos G, Ffrench RA et al (2005) Intrahepatic and peripheral blood virus-specific cytotoxic T lymphocyte activity is associated with a response to combination IFN-alpha and ribavirin treatment among patients with chronic hepatitis C virus infection. J Viral Hepat 12:125–129

Grakoui A, Shoukry NH, Woollard DJ et al (2003) HCV persistence and immune evasion in the absence of memory T cell help. Science 302:659–662

Grebely J, Conway B, Raffa JD et al (2006) Hepatitis C virus reinfection in injection drug users. Hepatology 44:1139–1145

Groux H, Bigler M, de Vries JE et al (1996) Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. J Exp Med 184:19–29

Groux H, O'Garra A, Bigler M et al (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389:737–742

Gruner NH, Gerlach TJ, Jung MC et al (2000) Association of hepatitis C virus-specific CD8+ T cells with viral clearance in acute hepatitis C. J Infect Dis 181:1528–1536

Guidotti LG, Chisari FV (2001) Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol 19:65–91

Harcourt G, Hellier S, Bunce M et al (2001) Effect of HLA class II genotype on T helper lymphocyte responses and viral control in hepatitis C virus infection. J Viral Hepat 8:174–179

Hashem M, El-Karaksy H, Shata MT et al (2011) Strong hepatitis C virus (HCV)-specific cell-mediated immune responses in the absence of viremia or antibodies among uninfected siblings of HCV chronically infected children. J Infect Dis 203:854–861

He XS, Rehermann B, López-Labrador FX et al (1999) Quantitative analysis of hepatitis C virus-specific CD8(+) T cells in peripheral blood and liver using peptide-MHC tetramers. Proc Natl Acad Sci USA 96:5692–5697

Houghton M, Abrignani S (2005) Prospects for a vaccine against the hepatitis C virus. Nature 436:961–966

Kamal SM, Fehr J, Roesler B et al (2002) Peginterferon alone or with ribavirin enhances HCV-specific CD4 T-helper 1 responses in patients with chronic hepatitis C. Gastroenterology 123:1070–1083

Kamal SM, Amin A, Madwar M et al (2004) Cellular immune responses in seronegative sexual contacts of acute hepatitis C patients. J Virol 78:12252–12258

Kantzanou M, Lucas M, Barnes E et al (2003) Viral escape and T cell exhaustion in hepatitis C virus infection analysed using Class I peptide tetramers. Immunol Lett 85:165–171

Kennedy PT, Urbani S, Moses RA et al (2006) The influence of T cell cross-reactivity on HCV-peptide specific human T cell response. Hepatology 43:602–611

Koziel MJ, Wong DK, Dudley D et al (1997) Hepatitis C virus-specific cytolytic T lymphocyte and T helper cell responses in seronegative persons. J Infect Dis 176:859–866

Kuntzen T, Timm J, Berical A et al (2007) Viral sequence evolution in acute hepatitis C virus infection. J Virol 81:11658–11668

Lafarge S, Hamzeh-Cognasse H, Chavarin P et al (2007) A flow cytometry technique to study intracellular signals NF-kappaB and STAT3 in peripheral blood mononuclear cells. BMC Mol Biol 8:64

Lai ME, Mazzoleni AP, Argiolu F et al (1994) Hepatitis C virus in multiple episodes of acute hepatitis in polytransfused thalassaemic children. Lancet 343:388–390

Lauer GM, Walker BD (2001) Hepatitis C virus infection. N Engl J Med 345:41–52

Lauer GM, Barnes E, Lucas M et al (2004) High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. Gastroenterology 127:924–936

Lechner F, Wong DK, Dunbar PR et al (2000) Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 191:1499–1512

Lucas M, Ulsenheimer A, Pfafferot K et al (2007) Tracking virus-specific CD4+ T cells during and after acute hepatitis C virus infection. PLoS One 2:e649

MacDonald AJ, Duffy M, Brady MT et al (2002) CD4 T helper type 1 and regulatory T cells induced against the same epitopes on the core protein in hepatitis C virus-infected persons. J Infect Dis 185:720–727

Maheshwari A, Ray S, Thuluvath PJ (2008) Acute hepatitis C. Lancet 372:321–332

Major ME, Mihalik K, Puig M et al (2002) Previously infected and recovered chimpanzees exhibit rapid responses that control hepatitis C virus replication upon rechallenge. J Virol 76:6586–6595

Martin B, Hennecke N, Lohmann V et al (2014) Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. J Hepatol 61:538–543

McKiernan SM, Hagan R, Curry M et al (2004) Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. Hepatology 40:108–114

Mehta SH, Cox A, Hoover DR et al (2002) Protection against persistence of hepatitis C. Lancet 359:1478–1483

Minton EJ, Smillie D, Neal KR et al (1998) Association between MHC class II alleles and clearance of circulating hepatitis C virus. Members of the Trent Hepatitis C Virus Study Group. J Infect Dis 178:39–44

Minutello MA, Pileri P, Unutmaz D et al (1993) Compartmentalization of T lymphocytes to the site of disease: intrahepatic CD4+ T cells specific for the protein NS4 of hepatitis C virus in patients with chronic hepatitis C. J Exp Med 178:17–25

Neumann-Haefelin C, Blum HE, Chisari FV et al (2005) T cell response in hepatitis C virus infection. J Clin Virol 32:75–85

Neumann-Haefelin C, McKiernan S, Ward S et al (2006) Dominant influence of an HLA-B27 restricted CD8+ T cell response in mediating HCV clearance and evolution. Hepatology 43:563–572

Neumann-Haefelin C, Spangenberg HC, Blum HE et al (2007) Host and viral factors contributing to CD8+ T cell failure in hepatitis C virus infection. World J Gastroenterol 13:4839–4847

Page K, Hahn JA, Evans J et al (2009) Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. J Infect Dis 200:1216–1226

Penna A, Pilli M, Zerbini A et al (2007) Dysfunction and functional restoration of HCV-specific CD8 responses in chronic hepatitis C virus infection. Hepatology 45:588–601

Pilli M, Zerbini A, Penna A et al (2007) HCV-specific T-cell response in relation to viral kinetics and treatment outcome (DITTO-HCV project). Gastroenterology 133:1132–1143

Post JJ, Pan Y, Freeman AJ et al (2004) Clearance of hepatitis C viremia associated with cellular immunity in the absence of seroconversion in the hepatitis C incidence and transmission in prisons study cohort. J Infect Dis 189:1846–1855

Prince AM, Brotman B, Huima T et al (1992) Immunity in hepatitis C infection. J Infect Dis 165:438–443

Prlic M, Lefrancois L, Jameson SC (2002) Multiple choices: regulation of memory CD8 T cell generation and homeostasis by interleukin (IL)-7 and IL-15. J Exp Med 195:F49–F52

Quiroga JA, Llorente S, Castillo I et al (2006) Cellular immune responses associated with occult hepatitis C virus infection of the liver. J Virol 80:10972–10979

Radziewicz H, Ibegbu CC, Fernandez ML et al (2007) Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression. J Virol 81:2545–2553

Razvi S, Schneider L, Jonas MM et al (2001) Outcome of intravenous immunoglobulin-transmitted hepatitis C virus infection in primary immunodeficiency. Clin Immunol 101:284–288

Riviere Y, Montange T, Janvier G et al (2012) Hepatitis C virus-specific cellular immune responses in individuals with no evidence of infection. Virol J 9:76

Rosen HR, Miner C, Sasaki AW et al (2002) Frequencies of HCV-specific effector CD4+ T cells by flow cytometry: correlation with clinical disease stages. Hepatology 35:190–198

Rushbrook SM, Ward SM, Unitt E et al (2005) Regulatory T cells suppress in vitro proliferation of virus-specific CD8+ T cells during persistent hepatitis C virus infection. J Virol 79:7852–7859

Sarobe P, Lasarte JJ, Casares N et al (2002) Abnormal priming of CD4(+) T cells by dendritic cells expressing hepatitis C virus core and E1 proteins. J Virol 76:5062–5070

Scarselli E, Cerino A, Esposito G et al (1995) Occurrence of antibodies reactive with more than one variant of the putative envelope glycoprotein (gp70) hypervariable region 1 in viremic hepatitis C virus-infected patients. J Virol 69:4407–4412

Schirren CA, Jung MC, Gerlach JT et al (2000) Liver-derived hepatitis C virus (HCV)-specific CD4(+) T cells recognize multiple HCV epitopes and produce interferon gamma. Hepatology 32:597–603

Schulze zur Wiesch J, Lauer JM, Day CL et al (2005) Broad repertoire of the CD4+ Th cell response in spontaneously controlled hepatitis C virus infection includes dominant and highly promiscuous epitopes. J Immunol 175:3603–3613

Scognamiglio P, Accapezzato D, Casciaro MA et al (1999) Presence of effector CD8+ T cells in hepatitis C virus-exposed healthy seronegative donors. J Immunol 162:6681–6689

Semmo N, Klenerman P (2007) CD4+ T cell responses in hepatitis C virus infection. World J Gastroenterol 13:4831–4838

Semmo N, Krashias G, Willberg C et al (2007) Analysis of the relationship between cytokine secretion and proliferative capacity in hepatitis C virus infection. J Viral Hepat 14:492–502

Shedlock DJ, Shen H (2003) Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science 300:337–339

Shoukry NH, Grakoui A, Houghton M et al (2003) Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. J Exp Med 197:1645–1655

Söderholm J, Ahlén G, Kaul A et al (2006) Relation between viral fitness and immune escape within the hepatitis C virus protease. Gut 55:266–274

Spada E, Mele A, Berton A et al (2004) Multispecific T cell response and negative HCV RNA tests during acute HCV infection are early prognostic factors of spontaneous clearance. Gut 53:1673–1681

Strickland GT, El-Kamary SS, Klenerman P et al (2008) Hepatitis C vaccine: supply and demand. Lancet Infect Dis 8:379–386

Sun JC, Williams MA, Bevan MJ (2004) CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. Nat Immunol 5:927–933

Syriopoulou V, Nikolopoulou G, Daikos GL et al (2005) Mother to child transmission of hepatitis C virus: rate of infection and risk factors. Scand J Infect Dis 37:350–353

Takaki A, Wiese M, Maertens G et al (2000) Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. Nat Med 6:578–582

Tatsumi T, Takehara T, Miyagi T et al (2011) Hepatitis C virus-specific CD8+ T cell frequencies are associated with the responses of pegylated interferon-alpha and ribavirin combination therapy in patients with chronic hepatitis C virus infection. Hepatol Res 41:30–38

Tester I, Smyk-Pearson S, Wang P et al (2005) Immune evasion versus recovery after acute hepatitis C virus infection from a shared source. J Exp Med 201:1725–1731

Thimme R, Oldach D, Chang KM et al (2001) Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 194:1395–1406

Thomson EC, Nastouli E, Main J (2009) Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV. AIDS 23:89–93

Thursz M, Yallop R, Goldin R et al (1999) Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. Lancet 354:2119–2124

Timm J, Lauer GM, Kavanagh DG et al (2004) CD8 epitope escape and reversion in acute HCV infection. J Exp Med 200:1593–1604

Ulsenheimer A, Gerlach JT, Gruener NH et al (2003) Detection of functionally altered hepatitis C virus-specific CD4 T cells in acute and chronic hepatitis C. Hepatology 37:1189–1198

Urbani S, Amadei B, Fisicaro P et al (2006) Outcome of acute hepatitis C is related to virus-specific CD4 function and maturation of antiviral memory CD8 responses. Hepatology 44:126–139

Waggoner SN, Hall CH, Hahn YS (2007) HCV core protein interaction with gC1q receptor inhibits Th1 differentiation of CD4+ T cells via suppression of dendritic cell IL-12 production. J Leukocyte Biol 82:1407–1419

Wedemeyer H, He XS, Nascimbeni M et al (2002) Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 169:3447–3458

Weiner Paliard X, Selby MJAJ et al (2001) Intrahepatic genetic inoculation of hepatitis C virus RNA confers cross-protective immunity. J Virol 75:7142–7148

Welsh RM, Selin LK (2002) No one is naive: the significance of heterologous T-cell immunity. Nat Rev Immunol 2:417–426

Yi JS, Cox MA, Zajac AJ (2010) T-cell exhaustion: characteristics, causes and conversion. Immunology 129:474–481

Zeremski M, Shu MA, Brown Q et al (2009) Hepatitis C virus-specific T-cell immune responses in seronegative injection drug users. J Viral Hepat 16:10–20

Zhang X, Dou J, Germann MW (2009) Characterization of the cellular immune response in hepatitis C virus infection. Med Res Rev 29:843–866