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TOPIC HIGHLIGHT

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Adaptive immune response during hepatitis C virus infection

Juan Ramón Larrubia, Elia Moreno-Cubero, Megha Uttam Lokhande, Silvia García-Garzón, Alicia Lázaro, Joaquín Miquel, Cristian Perna, Eduardo Sanz-de-Villalobos

Juan Ramón Larrubia, Elia Moreno-Cubero, Megha Uttam Lokhande, Silvia García-Garzón, Alicia Lázaro, Joaquín Miquel, Cristian Perna, Eduardo Sanz-de-Villalobos, Translational Hepatology Unit, Guadalajara University Hospital, University of Alcalá, E-19002 Guadalajara, Spain
Juan Ramón Larrubia, Department of Medicine and Medical Specialities, School of Medicine, University of Alcalá, E-28801 Alcalá de Henares, Spain
Author contributions: Larrubia JR contributed towards the conception and design of the review; Larrubia JR, Moreno-Cubero E and Lokhande MU co-wrote and Larrubia JR revised the manuscript; García-Garzón S, Lázaro A, Miquel J, Perna C and Sanz-de-Villalobos E contributed equally to the supportive work and supervision.
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Correspondence to: Juan Ramón Larrubia, MD, MSc, PhD, Translational Hepatology Unit, Guadalajara University Hospital, University of Alcalá, Donante de Sangre st, E-19002 Guadalajara, Spain. juan.larrubia@uah.es
Telephone: +34-949-909200 Fax: +34-949-209256
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Abstract

Hepatitis C virus (HCV) infection affects about 170 million people worldwide and it is a major cause of liver cirrhosis and hepatocellular carcinoma. HCV is a hepatotropic non-cytopathic virus able to persist in a great percentage of infected hosts due to its ability to escape from the immune control. Liver damage and disease progression during HCV infection are driven by both viral and host factors. Specifically, adaptive immune response carries out an essential task in controlling non-cytopathic viruses because of its ability to recognize infected cells and to destroy them by cytopathic mechanisms and to eliminate the virus by non-cytolytic machinery. HCV is able to impair this response by several means such as developing escape mutations in neutralizing antibodies and in T cell receptor viral epitope recognition sites and inducing HCV-specific cytotoxic T cell anergy and deletion. To impair HCV-specific T cell reactivity, HCV affects effector T cell regulation by modulating T helper and Treg response and by impairing the balance between positive and negative co-stimulatory molecules and between pro- and anti-apoptotic proteins. In this review, the role of adaptive immune response in controlling HCV infection and the HCV mechanisms to evade this response are reviewed.

Key words: Hepatitis C; Adaptive immune response; Hepatitis C virus-specific cytotoxic T cells; Hepatitis C virus-specific T helper cells; T regs; Hepatitis C virus escape mutations; Anergy; Apoptosis; Chemotaxis

Core tip: In the last few years, the knowledge about the role of adaptive immune response in hepatitis C pathogenesis has increased exponentially. This review summarizes our current understanding of the role of antigen-specific responses in hepatitis C virus (HCV) control and liver damage and discusses recent findings that identify costimulatory molecules modulation, apoptosis induction and chemokine regulation as major HCV mechanisms to evade immune control.

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INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic non-cytopathic virus which is able to evade immune system efficiently as mechanism to persist in infected hosts. To fight against a viral infection the host displays two kinds of immune responses, the innate and the adaptive immune response. The innate response is the first immunological barrier and it is essential in controlling cytopathic viruses but not enough in non-cytopathic infections. This primary response limits viral spreading but also acts as adaptive response activator through antigen presentation to viral specific cells. Adaptive response is the second line in the immunological defense and it plays a major role in non-cytopathic viral infections due to the ability of this kind of infections to remain occult to the innate system. The current knowledge about the role of the adaptive response role in viral control and pathogenesis during HCV infection will be reviewed in the following paragraphs.

GENERAL FEATURES OF ADAPTIVE IMMUNE RESPONSE

Non-cytopathic viruses have developed evolutionary mechanisms to remain hidden to the immune system, which is an advantage for their persistence. They are usually not highly infectious but produce long-lasting diseases that allow them to spread the infection over time. The host/non-cytopathic-virus relationship is a dynamic process in which the virus tries to decrease its visibility, whereas the host attempts to prevent and eradicate infection with minimal collateral damage to itself[1].

To control non-cytopathic viral infections, the activation of the adaptive immune system, especially the cellular immune response, is necessary (Figure 1). Naïve specific $CD4^+$ and $CD8^+$ T cells are primed by dendritic cells in the lymph nodes. Once these cells become activated, they change their phenotype into effector cells and migrate to the infected tissue attracted by the chemokines produced by the parenchymal cells. Primed specific $CD4^+$ cells are essential to allow the adequate activation of specific cytotoxic T cells by secretion of T helper (Th)-1 cytokines[2]. Subsequently, these specific cytotoxic T lymphocytes (CTL) play a major role in resolution of spontaneous infection because they are able to recognize the infected cells and destroy them by cytolytic mechanisms. On the other hand, they also produce type-1 cytokines that eliminate the virus without tissue damage. Both $CD4^+$ and $CD8^+$ cell activation depends on the engagement between T cell receptor and the Major Histocompatibility Complex (MHC)/epitope complex as well as the interaction between co-stimulatory molecules with their ligands and the adequate cytokine milieu[3]. When these cells have finished their effector task, they express negative co-stimulatory molecules and pro-apoptotic factors to switch-off their activity, and a subsequent constriction in the specific T cell population is produced. After this event, a memory T cell population is maintained for years to come to respond faster to a new infection and in certain cases to keep under control occult viral infection[4].

ADAPTIVE IMMUNE RESPONSE IN HCV INFECTION

The definitive barrier to control HCV infection is the adaptive immunity. This response has two arms to fight against pathogens; humoral and cellular immune response. Humoral immune response that means neutralizing and non-neutralizing antibodies (Non-nAbs), can endorse antiviral activity[5]. Cellular immune response shows antiviral immunity by means of virus specific CTLs and CD4 T helper cells, which play key effector and regulatory roles respectively. These T cells take part in viral clearing and pathogenesis of HCV by direct killing of infected cells or producing soluble factors that are able to clear the virus in a non-cytolytic manner, it also can lead to HCV pathogenic events, favoring direct liver damage and attracting non-specific inflammatory cells to perpetuate the liver inflammation[5].

Humoral immune response

nAbs generally play a critical role for controlling initial viremia and protecting from re-infection in viral infections. However, the role of the humoral immune response in the clearance of HCV infection is a controversial issue and there are still gaps in the knowledge of the interplay between HCV and nAbs. Antibodies generated during acute infection may be targeted against epitopes within structural and non-structural viral proteins; however, the majority of nAb have been mapped to the envelope glycoproteins E1 and E2[6].

HCV can be controlled in patients with hypogammaglobulinemia[7], suggesting this fact as a minor role for nAb in HCV control. In this situation, HCV-specific T cells may compensate for lack of neutralizing antibodies to obtain HCV clearance. Few studies supports the idea that HCV clearance occurs mostly in the absence of nAbs and these Abs alone are inadequate to eradicate HCV[8-12]. therefore, these data also confer a minor role to nAb in HCV control, although other studies propose, at least in some cases, a more important role for nAb in HCV clearing. In fact, there are also proofs supporting a maior role of nAb to neutralize virus infectivity during acute infection. The earliest studies in chimpanzees showed that hyperimmune serum agaist hypervariable region-1 induced protection against homologous HCV infection[13]. Evidence that nAb could protect from natural infection in humans arose form a cohort of women accidentally exposed to the same HCV-strain. In this cohort rapid induction of nAb during the early phase of infection contributed to HCV control[14]. Therefore, rapid

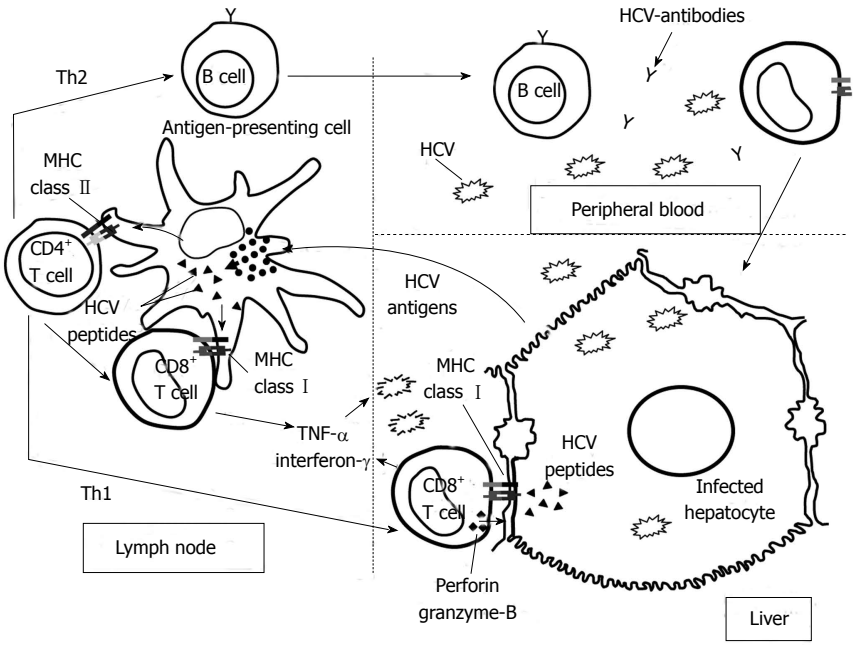

Figure 1 Hepatitis C virus-specific immune response activation. Graph showing the priming of naïve T cells by professional antigen-presenting cells in the lymph nodes after antigen up-take in the liver. After specific-T cell activation, these cells become effector T helper (Th) and cytotoxic T cells (CTL) and they migrate into the liver. Th2 response regulates B cells while Th1 response controls CTLs effector function. Specifc-CTLs are able to destroy hepatitis C virus (HCV) by cytolytic and non-cytolytic mechanisms.

induction of nAb early during infection is associated with spontaneous recovery and these antibodies appear to be more cross-neutralizing[15]. Nevertheless, these nAb are not detected in most of acute infections, while delayed appearance of high titer cross reactive nAbs in chronically infected patients suggests that selective mechanisms may operate to prevent the appearance of these Abs during acute infection[11]. Perhaps, the long-term persistence of these nAbs in chronically infected patients may regulate viral replication. In any case, delayed and inefficient neutralizing antibody response during chronic infection induces HCV escape mutations at Abs recognition sites[16], causing the selection of viral mutants.

Moreover, it has been proposed that HCV stimulates B cells in a B cell receptor-independent manner during chronic infection[17] and may favor the development of lymphoproliferative and autoimmune diseases[5]. Immune complexes induced during HCV infection are believed to play a pathogenetic role in the development of manifestations such as cryoglobulinemia, glomerulonephritis, porphyria cutanea tarda, and necrotizing cutaneous vasculitis[18-20].

Cellular immune response

A strong, multispecific and long-lasting T-cell immune response plays an important role for control of viral infection[8,21]. This efficient response has been observed both in patients as well as experimental models controlling HCV infection[10,12]. Efficiency of antiviral CTL response depends on where these cells are primed. Efficient antiviral CTL response is observed when it is primed in lymphoid organs, whereas within the liver, priming tends to induce T cell inactivation, tolerance or apoptosis[5]. Effector T cells fail to control persistant HCV due to multiple causes, such as: HCV escape mutation at T cell receptor recognition site, immunosuppressive effects exertion, Tregs induction, or T effector exhaustion or deletion[22-24].

Adaptive cellular response during acute HCV infection: Vigorous $CD4^+$ and $CD8^+$ T cell responses targeting multiple HCV regions with intrahepatic production of interferon (IFN)-γ emerge in acute hepatitis C infection[10,24-26]. The appearance of HCV-specific T cells can be detectable in the peripheral blood or in the liver compartment several weeks after infection in humans or in experimental chimpanzee models[8,27], in association to primary peak of transaminases. Moreover, HCV titer is reduced when these cells start producing IFN-γ and correlates with transaminases level decrease[27] (Figure 2).

The protective function of $CD4^+$ T cells appears to be due to the production of antiviral cytokines, but also due to their helping nature to antiviral B and $CD8^+$ T cells. The HCV clearance has been observed and correlated with vigorous proliferation of specific $CD4^+$ T cells[28,29] with concurrent interleukin (IL)-2 and IFN-γ production[30,31]. The early, sustained development of $CD4^+$ T cell response needs to be successful for viral clearance[31], whereas HCV-specific $CD4^+$ T cell responses are not observed in chronic HCV infection. Moreover, the recurrent viremia has been correlated with loss of previous strong $CD4^+$ T cell responses after several months of viral clearance[32,33]. CD4 help during acute HCV infection is essential to develop a spontaneous recovery[34], since CTL priming in presence of CD4 help is a critical factor in developing a protective response[31].

On the other hand, the magnitude of $CD8^+$ T cells response in acute HCV infection does not correlate with the clinical or viral outcome[31,35,36]. Expression of a dysfunctional phenotype with weak proliferation, IFN-γ production, cytotoxicity and increased levels of well known exhausted phenotype- programmed death-1 receptor (PD-1) are found in HCV infection, irrespective of infection progression[37-41]. Antigen-dependent reactivity of HCV-specific $CD8^+$ T cells has been proved during the early phase of acute infection associated with sub-

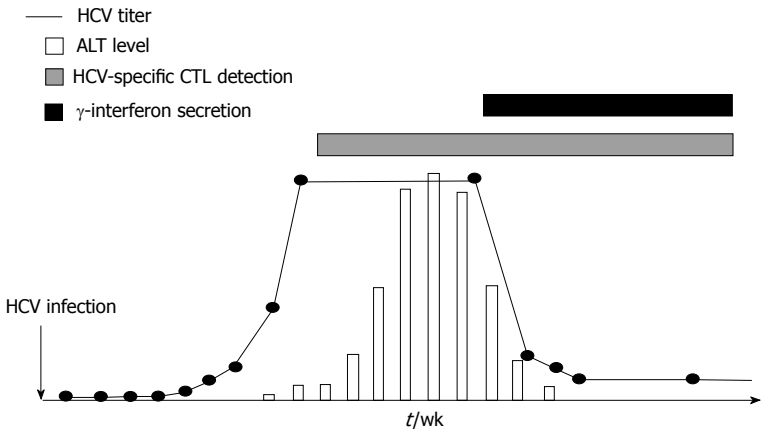

Figure 2 Cytolytic and non-cytolytic mechanisms to eliminate hepatitis C virus by specific cytotoxic T cells. Scheme showing the hepatitis C virus (HCV) viral load and alanine-amminotransferase (ALT) dynamics after acute HCV infection in relation to appearance of HCV-specific cytotoxic T cells and gamma-interferon secretion. Once HCV-specific cytotoxic T cells are detectable an ALT peak is observed, while when gamma interferon is secreted HCV titers decreased and ALT value becomes normal. CTL: Cytotoxic T lymphocyte.

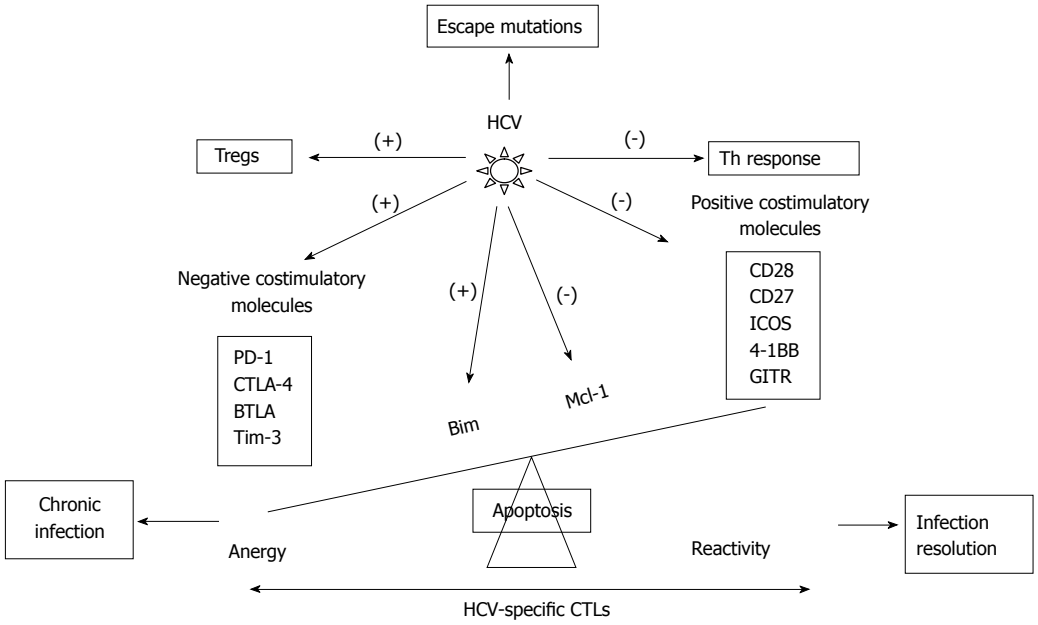

Figure 3 Scheme showing the hepatitis C virus strategies to escape from hepatitis C virus-specific cytotoxic T cells control. Hepatitis C virus (HCV) modulates the balance between positive and negative co-stimulatory molecules, between pro- and anti-apoptotic molecules, and between Th and Treg cells and develops escape mutations at TCR recognition site. PD-1: Programmed cell death protein 1; CTLA-4: Cytotoxic T-lymphocyte antigen 4; BTLA: B- and T-lymphocyte attenuator; Tim-3: T-cell immunoglobulin domain and mucin domain 3; ICOS: Inducible T-cell Costimulator; GITR: Glucocorticoid induced tumor necrosis factor receptor family related gene; Bim: Bcl-2-interacting mediator; Mcl-1: Myeloid leukemia cell differentiation protein; (-) inhibition; (+) induction.

sequent rapid decay of $CD8^+$ T cell responses in treatment responders, when these patients were submitted to antiviral therapy[42]. However, the development of self-sustaining memory T cells ($CD127^+$ HCV-specific $CD8^+$ T and $CD4^+$ T cells) are associated with HCV infection control[10,24,31]. In fact, years following sustained virologic response after anti-HCV treatment; it is possible to find HCV traces in association with HCV-specific T cell reactivity. These data suggest that HCV-specific memory T cells are essential to control HCV reservoirs completely in treated patients after the initial treatment induced viral control[43].

Adaptive cellular response during chronic HCV infection: Patients controlling HCV infection exhibit broader CTL responses with higher functional avidity and wider cross-recognition ability than patients with persistent HCV infection[44]. The main reasons for HCV persistence are the appearance of rapid HCV escape mutations[45,46], T cell exhaustion[47], and deletion[48], immune regulatory cytokines secretion[49] and immune modulatory T reg cell induction[50] (Figures 3 and 4).

HCV polymerase has high replication rate and lack of proof-reading capacity, which permits a rapid viral escape from emerging humoral and cellular immune responses, leading to persistent infection[45,51]. Mutation development in MHC class I restricted epitopes targeted by $CD8^+$ T cells are associated with persistence[52,53], which proved indirectly that MHC-restricted $CD8^+$ T cells exert selection pressure. Selection of viral escape mutations could occur early in acute infection and remained fixed thereafter, indicating that viral escape may indeed be a premature causative mechanism of $CD8^+$ T cell failure and viral persistence[54,55]. Furthermore, the MHC alleles can influence infection outcome; protective T cell responses target epitopes that can not allow escape mutations due to high

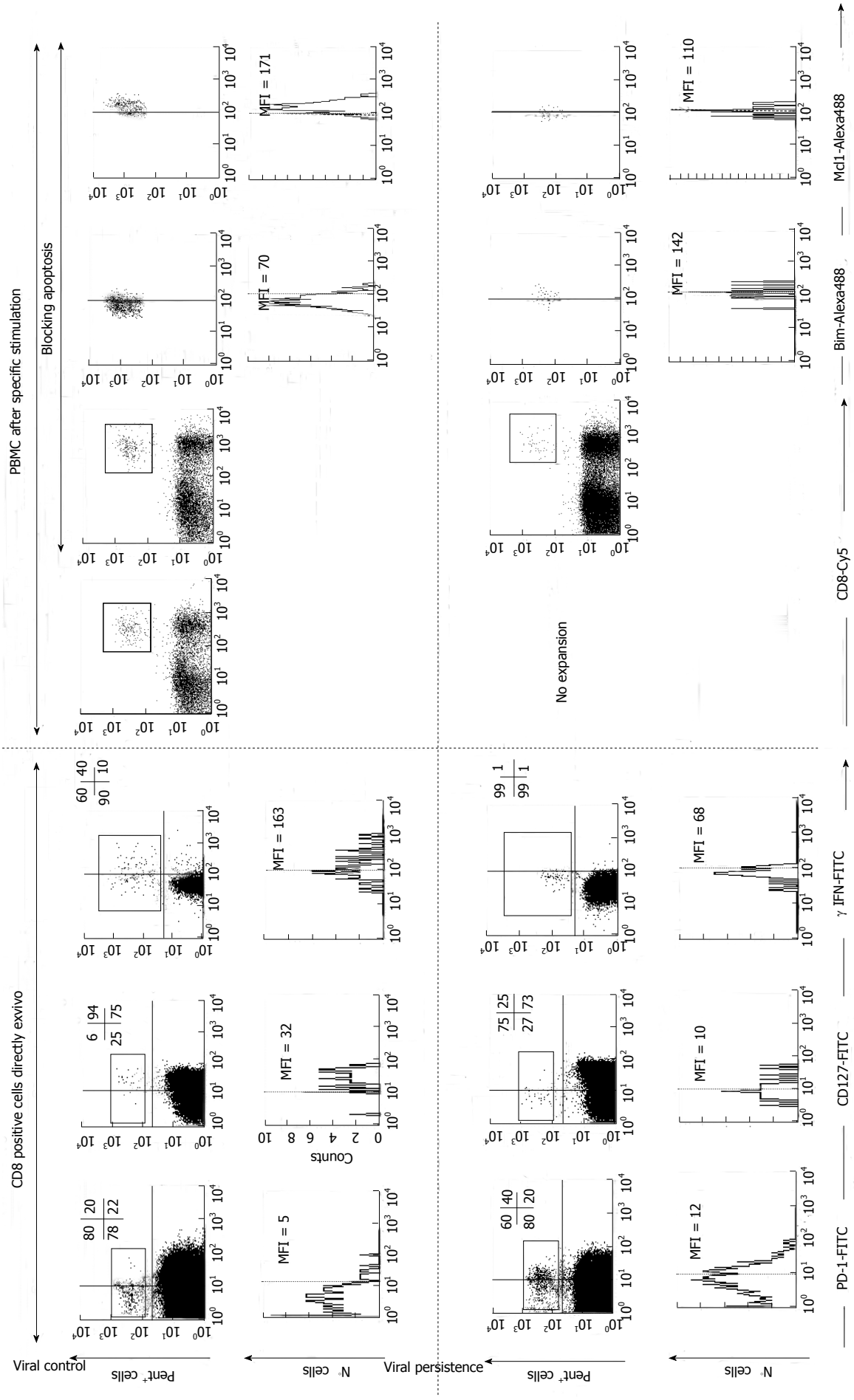

Figure 4 FACS® dot-plots and histograms of hepatitis C virus-specific CD8⁺ cells from hepatitis C virus patients with different viral control. Hepatitis C virus (HCV)-specific CD8⁺ cells were stained with Abs anti γ-IFN, anti-PD-1, anti-CD127, anti-Mcl-1, anti-Bim, anti-CD8 plus pentameric HLA-A2/NS3$_{1406}$ peptide complexes. PD1, γ-IFN and CD127 was analyzed directly *ex vivo*. Antigen specific proliferation was assessed after blocking or not apoptosis. After expansion Bim and Mcl-1 expression was analyzed. HCV-specific CTLs controlling HCV infection expressed a CD127^{high}, PD-1^{low}, Mcl-1^{high} and Bim^{low} phenotype while CTLs not controlling HCV displayed the opposite phenotype. MFI: mean fluorescence intensity, Pent: Pe-HLA-A2/NS3$_{1406}$ pentameric complexes. PBMC: Peripheral blood mononuclear cells. PD-1: Programmed cell death protein 1; IFN: Interferon; Bim: Bcl-2-interacting mediator; Mcl-1: Myeloid leukemia cell differentiation protein.

costs for viral replicative fitness[56-59]. In fact, there is an association between expression of some MHC class-I molecules, such as HLA-B27, and protection following HCV infection. This is explained because mutations in immunodominant epitopes recognized by these MHC class- I molecules lead to substantial viral loss of fitness or because a cluster of mutations is needed to impair T cell recognition.

The secretion of certain immune-regulatory cytokines is also related with HCV persistence. IL-10 cytokine is found to increase in chronic HCV infection[60]. In chronic HCV patients, the suppression of IFN-γ production and proliferation of virus-specific $CD4^+$ and $CD8^+$ T cells have been observed in livers with IL-10-producing HCV-specific $CD8^+$ T cells[61]. IL-10 produced by monocytes or natural killer (NK) cells also downregulates effector T cell responses. For instance, monocytes secrete IL-10 in response to HCV core-mediated Toll like receptor (TLR) 2 stimulation, *in vitro*[62]. IL-10 producing HCV-specific $CD8^+$ T cells inhibit IFN-α production[63], but also promote apoptosis of plasmocytoid dendritic cells[62]. In addition, intrahepatic HCV-specific IL-10 producing $CD8^+$ T cells prevent liver damage during chronic infection[64]. Moreover, tumor growth factor (TGF)-β is also involved in antiviral immune suppression and chronic HCV infection evolution[49]. Finally, HCV infection can also shift the ratio between T cell-sustaing cytokines such as IL-2[65]. To sum up these data, regulatory cytokines such as IL-10 or TGF-β develop a dual task. First of all, they impair T cell responses to allow viral persistence but also decrease liver damage to extend host survival.

Regulatory T cells (Tregs) are important to control the balance between host damage and viral control produced by specific immune response. In cases of excessive immune response that could be harmful for the host, these cells can induce immune-tolerance to the viral epitopes. The T cell subset with suppressive function, $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T (Treg) cells, engages in the control of auto-immunity and immune responses through various mechanisms including the inhibition of antigen presenting cell maturation and T-cell activation[66]. HCV infection increases the frequency of Treg cells and the extent of suppression irrespective of the outcome of the infection[67]. However, higher Tregs frequency has been observed in chronic HCV infected patients than in resolved cases[50,68-70]. Interestingly, depletion of $CD25^+$ cells enhance *in vitro* responsiveness of the remaining HCV-specific effector cells[50,68,69], which suggests a fundamental role of Tregs in the establishment of chronic HCV infection. Moreover, Treg cells are induced and proliferated in chronic HCV infection and appeared to alter liver inflammation[70]. Conversely, Programmed Death Ligand-1 mediated inhibition limits the expansion of Tregs by controlling STAT-5 phosphorylation (pSTAT-5)[71], which can diminish suppressive function of Tregs, leading to viral load control and ultimately ensuring long-lasting host survival.

On the other hand, HCV is able to induce the up-regulation of different negative co-stimulatory molecules in order to provoke an anergic status on HCV-specific T cells. These effector cells are featured by their inability to kill infected hepatocytes, and by the impairment of proliferation and production of type-1 cytokines after antigen recognition. These features are more intense in the intrahepatic comparment due to the higher antigenemia and the tolerogenic hepatic enviorment[72]. Overall, T cell exhaustion follows a predictable pattern. T cells that undergo exhaustion first lose their capacity to produce IL-2, a cytokine that supports proliferation. IL-2 is predominantly produced by $CD4^+$ T cells, whereas $CD8^+$ T cells produce little IL-2 themselves and depend on $CD4^+$ T cell help. This is followed by sequential loss of cytotoxicity and TNF-α and IFN-γ production[73].

PD-1 is one of that molecules involved in the generation of a state of exhaustion on HCV-specific $CD8^+$ T cells during chronic HCV infection[74]. Importance of PD-1 expression on HCV-specific T cell effector dysfunction has been described[75-77]. In addition, blocking of PD-1 signaling results in the functional restoration of blood-derived HCV-specific $CD8^+$ T cell responses in chronic infection[47,77]. However, to restore function of more exhausted HCV-specific T cells isolated from liver biopsies of infected patients, there is need of Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) blockade in addition to PD-1 blockade[78]. On top of this, the co-expression of other inhibitory receptors, besides PD-1 and CTLA-4, such as 2B4, CD160, KLRG1[79] and, T cell immunoglobulin and mucin domain containing molecule 3 (Tim-3)[80] occurred in about half of HCV-specific $CD8^+$ T cell responses and correlate with low or intermediate level of CD127 expression, impaired proliferative capacity, an intermediate T cell differentiation stage[81] (Figures 3 and 4). These data indicate that HCV infection modulates different negative co-stimulatory molecules to favor the development of HCV-specific $CD8^+$ T cell exhaustion. On the other hand, HCV infection is also able to regulate pro-apoptotic pathways to induce HCV-specific T cell deletion, in order to escape from immune response[82] (Figures 3 and 4). HCV-specific CTLs from chronic patients targeting the virus express an exhausted phenotype associated to the up-regulation of the pro-apoptotic molecule Bcl-2-interacting mediator (Bim) and down-regulation of induced myeloid leukemia cell differentiation protein (Mcl-1). Bim activity is blocked by the anti-apoptotic molecule Mcl-1. The reactivity of these cells is impaired due to the imbalance between Mcl-1 and Bim during chronic infection but can be restored by blocking apoptosis[48,75] (Figures 3 and 4). In Table 1 the phenotype and reactivity of peripheral HCV-specific CTLs according to viral control after modulating apoptosis and different co-stimulatory molecules is summarized.

Persistent HCV infection also affects $CD4^+$ T cell responses but can be only partially restored by PD-1/PD-L1 blockade or by antigen removal[83,84]. In chronic HCV infection, NK cells develop a regulatory function against $CD4^+$ T cells that may be involved in the antigen-specific defective T helper immunity[85,86].

Table 1 Summary of the phenotypic and functional features of hepatitis C virus-specific CD8$^+$ cells according to hepatitis C virus control directly *ex vivo* and after different *in vitro* treatments

	PD-1 *ex vivo*	CD127 *ex vivo*	Mcl-1 *ex vivo*/after TCR triggering	Bim *ex vivo*/after TCR triggering	Expansion[1]	Expansion (αPD-L1)[2]	Expansion (zVAD-fmk)[3]	Expansion (αPD-L1 and αCTLA-4)[4]	Expansion (αPD-L1 and α41BB)[5]
Persistent infection									
PBMC	(+)	(-)	Low/low	Low/high	Impaired	Restored	Restored	Restored	Restored
IHMC	(++)	(-)			Impaired			Restored	Impaired
Resolved infection									
PBMC	(-)	(++)	High/low	Low/low	Positive	Positive	Positive		

(-) Low expression; (+) Intermediate expression; (++) High expression. Expansion: proliferation after specific *in vitro* challenge [1]without any other treatment; [2]in presence of anti-PD-L1 mAb; [3]blocking apoptosis after treatment with z-VAD-fmk, in presence of anti-PD-L1 mAb and anti-CTLA4 mAb[4] and adding anti-PD-L1 mAb plus stimulating anti-41BB mAb[5]. PBMC: Peripheral blood mononuclear cells; IHMC: Intrahepatic mononuclear cells[47,48,75,76,78,105]. Bim: Bcl-2-interacting mediator; CTLA4: Cytotoxic T-Lymphocyte Antigen 4.

ACCEPTED MODEL OF HCV PATHOGENESIS

As previously commented, specific CTLs play a central role in HCV immunopathogenesis. These cells are not only able to kill some infected hepatocytes inducing a minor liver damage, but also they secrete type-I cytokines responsible for non-cytopathic virus clearing. To attract these cells into the liver, the infected hepatocytes secrete different chemokines. The migration of lymphocytes to the liver is a complex process including adhesion, rolling, and transendothelial migration. Chemokines and their receptors play an essential role in this multistep pathway[87]. During primo infection, when the adaptive immune system is not able to control infection, the infected hepatocytes continue secreting chemokines to try to attract more defensive cells. In viral chronic hepatitis, the expression of different chemokines in the liver has been described. CXCL-10 is increased in the liver and peripheral blood during chronic viral hepatitis[88,89]. This molecule is produced by hepatocytes and sinusoidal endothelial cells. Moreover, CXCL9 and CXCL11 are also increased in serum and liver of subjects with chronic viral hepatitis[90]. CXCL9 is detected primarily on sinusoidal endothelial cells, while CXCL-11 is produced mainly by hepatocytes[91]. CCL5 intrahepatic expression is also elevated in viral chronic hepatitis and is produced by hepatocytes, sinusoidal endothelial cells and biliary epithelium. Finally, several studies have reported an increased level of CCL3 and CCL4 either in the liver or in serum. These molecules are detected on endothelial cells, on some hepatocytes and biliary epithelial cells[92]. The expression of all these chemokines in the liver can be induced directly by viral proteins. Previous reports have shown a high hepatocyte synthesis of CXCL10, CXCL9 and CCL5, induced by some HCV proteins such as NS5A and core[93], although a recent *in vitro* study suggests that HCV proteins could also decrease CCL5 and CXCL10 genes expression[94]. All these chemokines recruit T cells with a Th1/Tc1 phenotype, expressing specific chemokine receptors such as CCR5 and CXCR3. The non-ELR-CXC chemokine attracts CXCR3 expressing T cells while CC chemokine attract CCR5 expressing T cells to the liver. Consequently, in viral chronic hepatitis, an intrahepatic enrichment of CCR5 and CXCR3 expressing T cells, located in hepatic lobule and portal tracts has been shown, while these populations are very infrequent in uninfected subjects[95] (Figure 5).

Persistent HCV infection is characterized by a non-specific inflammatory infiltrate in the liver, mainly of CD8$^+$ cells[96], responsible for liver damage. These cells are attracted by the interaction between the intrahepatic secreted chemokines and the chemokine receptors expressed on T cells. Actually, previous reports have shown a correlation between liver inflammation and liver infiltrating CXCR3/CCR5 expressing T cells. The frequency of these cells was positively correlated with portal and lobular inflammation. These data suggest that CCR5 and CXCR3 could play an important role in chronic liver damage by means of inflammatory T cells recruitment into the liver. Obviously, several previous studies have also shown a correlation between liver inflammation and chemokine levels. Intrahepatic CXCL10 mRNA levels are associated with intralobular inflammation[97] and also CXCL9 and CXCL11 correlate with the grade of liver inflammation[98]. Furthermore, CC chemokines are also correlated with the intrahepatic inflammatory activity[99]. Clearly, intrahepatic CCL5 level correlates with the inflammatory activity but not with liver fibrosis. Bearing in mind all the previous data, it is possible to speculate that chemokines are secreted in the infected liver to attract an adaptive immune response able to clear the virus. Unfortunately, when the specific response fails, these chemokines also attract non-specific inflammatory cells, which are not able to remove the virus but produce by-standard liver inflammation. Therefore, as chemokines are nonspecific chemoattractants, intrahepatic inflammatory infiltrate during chronic infection is mainly non-virus-specific and consequently unable to eliminate the infection, but able to produce cytokines capable of initiating and perpetuating hepatic damage and fibrogenesis[95, 100, 101] (Figure 6).

STRATEGIES TO RESTORE ADAPTIVE IMMUNE RESPONSE IN HCV INFECTION

Taking into account that specific T cell responses are essential to control HCV during natural immune response,

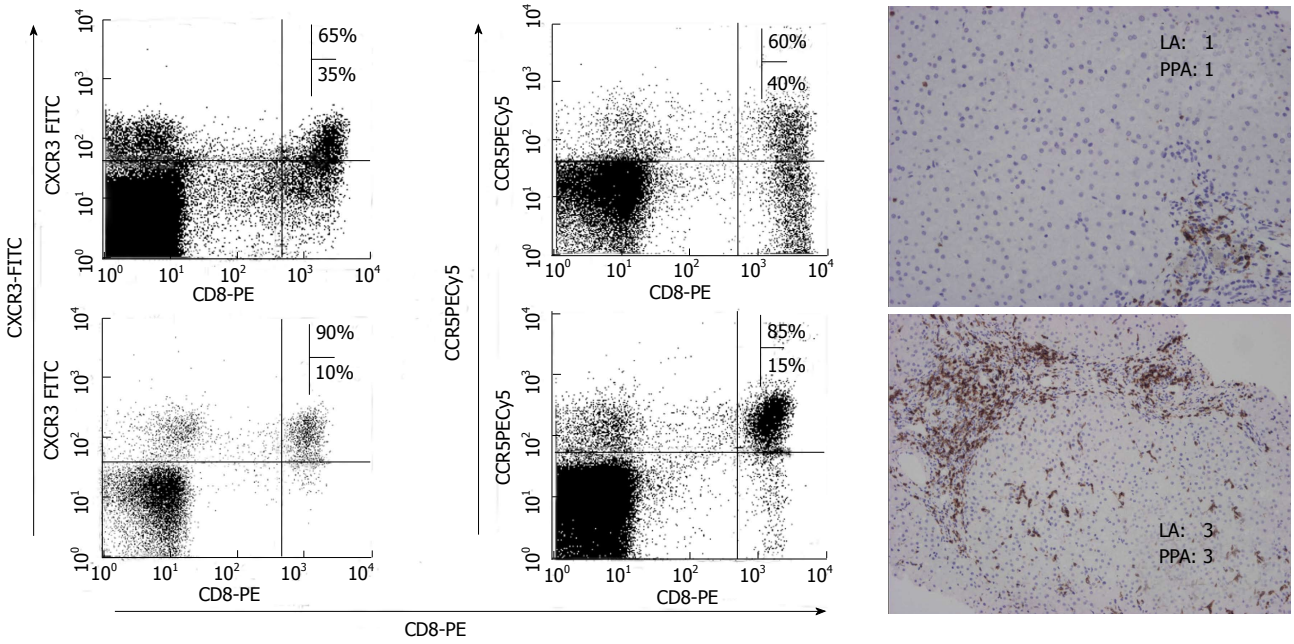

Figure 5 Chemokine receptor expression of liver infiltrating CD8 T cells according to liver damage. Immunohistochemical CD8 staining of liver samples from patients with different degree of liver inflammation. CCR5 and CXCR3 expression on CD8 T cells from those samples was studied by FACS® analysis after staining with the appropriate mAbs. A positive correlation between CCR5 and CXCR3 expression on intrahepatic total $CD8^+$ T cells and liver inflammation was observed. LA: Lobular activity; PPA: Peri-portal activity according to Metavir Index.

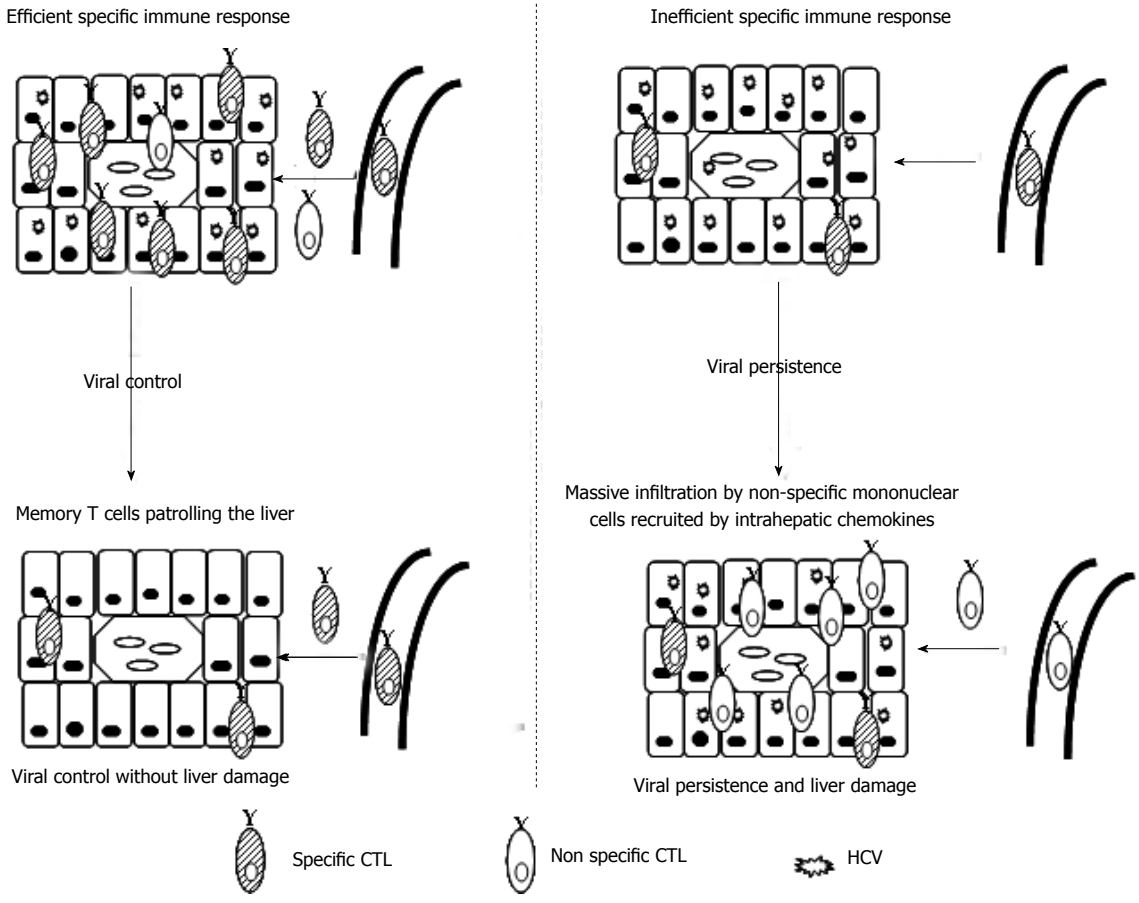

Figure 6 Scheme showing the role of T cell intrahepatic recruitment according to the degree of liver damage and viral control. In resolved hepatitis C virus (HCV) infection an adequate effector T cell response is attracted to the liver to clear the virus. After that, a memory T cell population is continuously patrolling the liver to keep under control viral traces. Nevertheless, in persistent infection, after HCV-specific T cell failure to control infection, a non-specific inflammatory infiltrate is sequestered into the liver, responsible of the persistent liver damage. CTL: Cytotoxic T lymphocytes.

several studies have been performed to analyze the role of different therapeutic approaches on T cell response to know whether it is possible to reverse dysfunction of these cells. One possible mechanism to restore T cell function could be to decrease exhaustion by viral load reduction. During HCV infection, a clear HCV-specific cytotoxic T cell response restoration during anti-HCV therapy has not been shown, as could be expected after treatment induced HCV load decrease[102,103], although specific T helper response restoration in sustained viral responders has been described[104]. Nevertheless, patients presenting a better HCV-specific CD8 cell proliferative potential at baseline, are more likely to present a rapid and sustained viral response. Therefore, currently there are contradictory data about the effect of HCV titter reduction on specific T cell response during chronic hepatitis treatment.

In any case, several pre-clinical studies using different strategies have been performed to try to restore HCV-specific responses *in vitro*. Modulation of co-stimulatory molecules (Table 1), in addition to blocking immunosuppressive cytokines could be promising strategies to restore an effective T cell response. The blockade of negative co-stimulatory molecules, such as PD-1, CTLA-4, Tim-3, has shown *in vitro* to increase specific-T cell reactivity. This strategy can be combined with the stimulation of positive co-stimulatory molecules such as 4-1BB[105]. Finally, after restoring a T cell response could be necessary to boost that response using a therapeutic vaccine[106]. Moreover, redirecting $CD8^+$ T cells to recognize HCV-epitopes by HCV-specific Vβ and Vα T cell receptor gene transduction has been demonstrated *in vitro* and it could be a future strategy to induce a new HCV-specific T cell population in chronic patients with deletion of these responses[107]. Although all these results seem to be quite promising, the blockade of negative co-stimulatory pathways, stimulation of positive ones and generation of new specific T cells could lead to the development of autoimmune or lymphoproliferative diseases, which could prevent the use of this strategy as a therapeutic tool in humans in the near future. Therefore, more research is necessary in this field before these strategies are suitable for the treatment of chronic viral infections[75,78,106].

CONCLUSION

HCV is a hepatotropic non-cytopathic virus able to produce a chronic liver disease. HCV clearing resides in the efficacy of adaptive immune response and, particularly HCV specific CTL response plays a central role in viral control through cytopathic and non-cytopathic mechanisms. Nevertheless, during persistent infection, specific-CTL response is impaired due to its exhaustion and deletion, emergence of HCV escape mutations and lack of T helper cooperation. Several *in vitro* strategies have shown to be effective in T cell response restoration but it is necessary to perform more research before these approaches can be applied to clinical practice. Finally, when the virus is not controlled by adaptive response a non-specific inflammatory infiltrate is attracted to the liver which is responsible for the persistent low-grade liver damage, allowing the generation of liver fibrosis and disease progression.

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