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World J Gastroenterol 2007 September 28; 13(36): 4839-4847
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

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Host and viral factors contributing to CD8+ T cell failure in hepatitis C virus infection

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Supported by the Deutsche Forschungsgemeinschaft (Emmy Noether Programm, SFB 610), the Wilhelm Sander Stiftung, and the Bundesministerium fuer Wissenschaft und Forschung (Start-up fonds Kompetenznetz Hepatitis)
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Received: June 26, 2007 Revised: July 9, 2007

Abstract

Virus-specific CD8+ T cells are thought to be the major anti-viral effector cells in hepatitis C virus (HCV) infection. Indeed, viral clearance is associated with vigorous CD8+ T cell responses targeting multiple epitopes. In the chronic phase of infection, HCV-specific CD8+ T cell responses are usually weak, narrowly focused and display often functional defects regarding cytotoxicity, cytokine production, and proliferative capacity. In the last few years, different mechanisms which might contribute to the failure of HCV-specific CD8+ T cells in chronic infection have been identified, including insufficient CD4+ help, deficient CD8+ T cell differentiation, viral escape mutations, suppression by viral factors, inhibitory cytokines, inhibitory ligands, and regulatory T cells. In addition, host genetic factors such as the host's human leukocyte antigen (HLA) background may play an important role in the efficiency of the HCV-specific CD8+ T cell response and thus outcome of infection. The growing understanding of the mechanisms contributing to T cell failure and persistence of HCV infection will contribute to the development of successful immunotherapeutical and -prophylactical strategies.

Key words: Hepatitis C virus; CD8+ T cells; T cell failure; Viral escape; Programmed death 1; Regulatory T cells; T cell maturation; Human leukocyte antigen

Neumann-Haefelin C, Spangenberg HC, Blum HE, Thimme R. Host and viral factors contributing to CD8+ T cell failure in hepatitis C virus infection. *World J Gastroenterol* 2007; 13(36): 4839-4847

http://www.wjgnet.com/1007-9327/13/4839.asp

INTRODUCTION

The host immune response to pathogens involves various components of the immune system, including innate, humoral, and cellular immunity, the latter consisting of CD4+ and CD8+ T cells. All components of the immune response might have distinct roles in the outcome and pathogenesis of HCV infection and will be discussed in separate reviews in this issue of *WJG*. In this review, we will focus on the CD8+ T cell response to HCV infection. CD8+ T cells recognize viral antigen presented by HLA class I molecules on professional antigen presenting cells (CD8+ T cell priming) and on infected target cells (e.g. hepatocytes). Their antiviral activity includes cytotoxicity as well as the secretion of antiviral cytokines such as interferon-gamma (IFN-γ). In the following, successful virus-specific CD8+ T cell responses associated with viral clearance as well as ineffective CD8+ T cell responses present in persistent HCV infection will be described. The main focus of this review, however, is the multiple mechanisms that contribute to CD8+ T cell failure and viral persistence.

CD8+ T CELL RESPONSE IN ACUTE HCV INFECTION

During acute resolving HCV infection, vigorous virus-specific CD8+ T cell responses that target multiple epitopes can be detected approximately 4-8 wk after infection, and their emergence is temporally associated with the onset of liver disease[1-4] (Figure 1A). However, the virus-specific CD8+ T cells are not able to secrete antiviral cytokines such as IFN-γ in this early phase of infection, a status referred to as 'stunned phenotype'[2,3]. In a later phase of infection, virus-specific CD8+ T cells regain their ability to secrete antiviral cytokines, and this is temporally associated with a rapid decline of viremia and finally viral clearance. Knowledge about the intrahepatic virus-specific CD8+ T cell response during acute HCV infection was obtained from experimentally infected chimpanzees. Responses accumulate in the liver 8-14 wk after infection and coincide with liver disease as well as viral clearance[5,6]. After resolution of infection, virus-specific CD4+ and

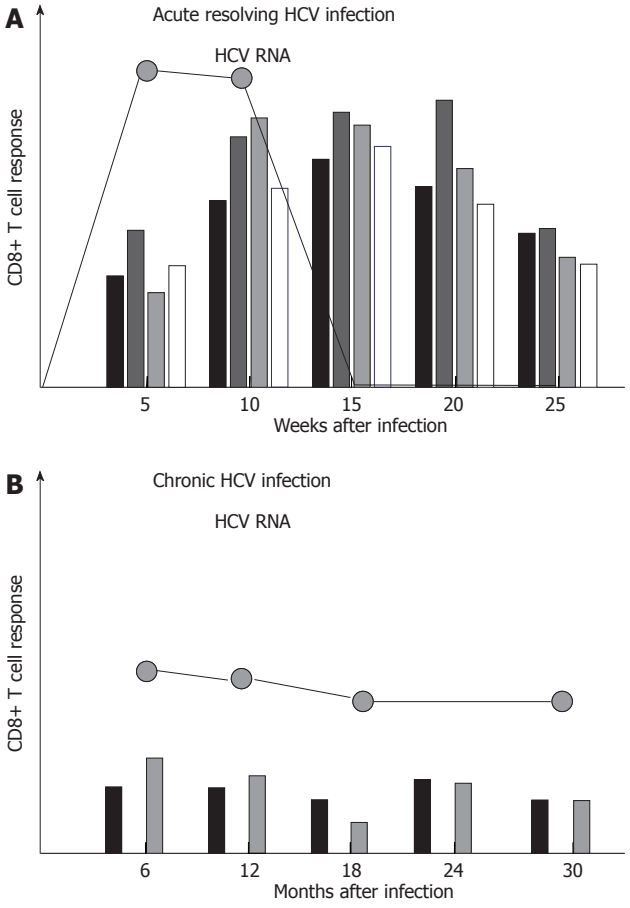

Figure 1 **A**: Virus-specific CD8+ T cell responses are strong, multi-specific, and sustained in acute resolving HCV infection; **B**: Virus-specific CD8+ T cell responses are weak and narrowly focused in chronic HCV infection.

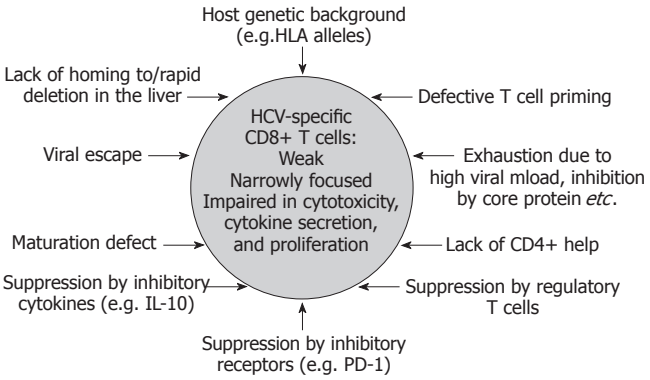

Figure 2 Possible Mechanisms of CD8+ T cell failure in persistent HCV infection.

CD8+ T cell responses persist for decades and can even outlast humoral responses[7]. Virus-specific CD8+ T cells also play a role in mediating protective immunity. Indeed, evidence for protective immunity comes from both, epidemiological studies as well as experimental studies[8]. Chimpanzees re-challenged by HCV showed a shorter period and lower level of viremia than naïve animals[9-11]. Sterilizing immunity against HCV, however, may not exist, since multiple episodes of heterologous or homologous re-infection have been observed in both, humans and chimpanzees.

In contrast to acute resolving HCV infection, the CD8+ T cell response in acute persisting HCV infection has been less defined. Previous reports comparing the CD8+ T cell response in acute resolving versus acute persisting HCV infection in chimpanzees and men found significantly weaker and more narrowly focused virus-specific CD8+ T cell responses in those subjects developing persistent infection[1,2,5,6]. More recent studies, however, could not confirm this finding[4,12,13]. For example, Cox *et al* performed a prospective longitudinal study in young iv drug users and analyzed the T cell response in 4 individuals with resolution of acute HCV infection and 15 individuals who progressed to chronic infection. Although all 4 individuals with resolving infection mounted virus-specific CD8+ T cell responses and those 4 individuals who lacked CD8+ T cell responses developed chronic infection, the CD8+ T cell response did not differ significantly between resolvers and persistently infected individuals[4]. Urbani *et al* studied 6 patients with acute resolving and 11 patients with acute persisting HCV infection and found an association between strong and multispecific CD4+, but not CD8+ T cells with viral clearance. However, patients developing chronic infection displayed prolonged CD8+ T cell dysfunctions and maturational defects[12]. This discordant role of CD4+ and CD8+ T cells was confirmed by Kaplan *et al*, albeit their analysis was limited to two HLA-A2 restricted CD8+ T cell epitopes[13].

In sum, acute resolving HCV infection is associated with strong, broadly directed and sustained CD8+ T cell responses, while a universal picture of the CD8+ T cell response in acute persistent HCV infection has not yet been defined.

CD8+ T CELL RESPONSE IN CHRONIC HCV INFECTION

In contrast to acute resolving infection, CD8+ T cell responses are usually weak or even absent in chronic HCV infection, targeting only few epitopes[14-22] (Figure 1B). In this context, it is important to point out that at least in some chronically infected patients, the CD8+ T cell response targets several epitopes[17,18,22]. Importantly, however, these HCV-specific CD8+ T cells display functional impairments, including reduced cytotoxicity, reduced secretion of antiviral cytokines such as IFN-γ, and a reduced proliferative capacity[23-25]. In addition, many CD8+ T cell responses do not target a present antigen, but rather a historical antigen due to viral escape (see below). Different mechanisms which might be involved in the failure of the HCV-specific CD8+ T cell response in persistent infection will be discussed in the following (Figure 2).

MECHANISMS OF CD8+ T CELL FAILURE

Primary failure and exhaustion

As discussed above, some patients with chronic HCV infection lack strong and multispecific CD8+ T cell responses, however, it is difficult to distinguish if virus-specific CD8+ T cell responses were not primed initially (primary CD8+ T cell failure) or responses were primed,

but vanished quickly (CD8+ T cell exhaustion). Results obtained from the early phase of acute HCV infection in chimpanzees and in health care workers infected through needle stick exposure support the hypothesis that CD8+ T cells are not primed at least in some patients with acute persisting HCV infection[2,5,6]. In a prospective longitudinal study of young iv drug users, however, CD8+ T cell exhaustion was indeed demonstrated for at least one targeted epitope in each subject developing chronic infection[4].

An impaired priming of HCV-specific CD8+ T cells might be mediated by numeric and functional impairments of antigen-presenting cells, e.g. macrophages and dendritic cells; however, this topic remains controversial[26-35].

CD8+ T cell exhaustion might be explained by general as well as HCV-specific mechanisms. Of note, it has been demonstrated in the lymphocytic choriomeningitis virus (LCMV) mouse model that high viral loads lead to an unresponsive state of virus-specific CD8+ T cells, downregulation of T cell receptors, and finally physical deletion of virus-specific CD8+ T cells[36-39]. Recent data indicate that the inhibitory receptor PD-1 might be involved in this process (see below), and it has been postulated that the detrimental effect of high viral load may not only apply in LCMV infection, but in different viral infections including HCV infection. Regarding HCV-specific mechanisms of CD8+ T cell exhaustion, the core protein has been reported to impair CD8+ T cell activation, e.g. through interaction with membrane-bound complement receptor gC1qR[40-42].

Lack of CD4+ help

While CD8+ T cells are considered the major effector cells against viral pathogens, the successful elimination of HCV might be highly dependent on sufficient CD4+ T cell help. Indeed, it has been demonstrated in the LCMV mouse model that CD4+ T cell help is needed to sustain cytotoxic CD8+ T cell responses during chronic viral infections[43]. In chronic HCV infection, however, CD4+ T cell responses are very weak or even absent and functionally impaired, e.g. secrete low amounts of IL-2[44,45]. Findings in the chimpanzee model support the central role of CD4+ help in CD8+ T cell mediated viral clearance. When CD4+ T cells were depleted by neutralizing antibodies prior to viral re-challenge, HCV viremia was prolonged, CD8+ escape variants were selected and HCV finally persisted[46]. Consistent with this concept, HCV-specific CD8+ T cell responses were seen almost exclusively in the face of a strong CD4+ T cell response in a study of acutely HCV infected patients[1]. A recent study demonstrated that the outcome of acute HCV infection was associated with efficient virus-specific CD4+ T cell responses. In this study, however, HCV-specific CD8+ T cell responses were induced irrespective of virological outcome or HCV-specific CD4+ T cell responses[13].

Suppression by regulatory T cells

In the last few years, the concept of regulatory T cells has undergone a comeback and different types of regulatory T cells have been characterized in different clinical settings. In HCV infection, the role of CD4+CD25+ Foxp3+ regulatory T cells as well as IL-10 producing CD8+ T cells has been defined. In chronically HCV infected patients, CD4+CD25+ T cells have been found in a higher frequency compared to individuals with resolved HCV infection or healthy controls[47-49]. These regulatory T cells suppress the proliferation as well as interferon-gamma secretion of virus-specific CD8+ T cells *in vitro*. The suppression by CD4+CD25+ T cells was cell-cell contact dependent[47,48], it was independent of suppressive cytokines such as IL-10 and TGF-β in some but not all studies[47,48,50]. Interestingly, the suppression was not restricted to HCV-specific CD8+ T cells, but also included CD8+ T cells specific for other viruses, such as EBV or influenza[47,50]. However, specificity *in vivo* might be mediated by the enrichment of CD4+CD25+ T cells in the liver[51]. While CD4+CD25+ T cells might limit immunopathology in the chronic phase of HCV infection[52], it has been suggested that they may facilitate viral persistence in the acute phase of infection. However, studies in larger cohorts of patients with acute HCV infection have not yet been reported. The induction of CD4+CD25+ regulatory T cells is still little characterized, however, they could be induced by certain HCV peptides from peripheral blood mononuclear cells (PBMCs) from HCV-infected, but not healthy individuals *in vitro*[53].

Another type of regulatory T cells in HCV infection is virus-specific regulatory CD8+ T cells that express high levels of IL-10. These regulatory T cells have been detected in the liver of HCV-infected individuals; they could be expanded upon stimulation with HCV epitope peptides and their suppression of virus-specific CD8+ effector T cells could be blocked by neutralizing IL-10 antibodies[54]. This virus-specific regulatory T cell population might have an important role in the prevention of liver damage during chronic HCV infection[55].

The spectrum of regulatory T cells involved in HCV infection may further expand, since we recently described the induction of regulatory CD8+ T cells from the PBMC of HCV-infected patients which also expressed high levels of FoxP3 and CD25[56]. A comprehensive review on the different types of regulatory T cells in HCV and HBV infection by Billerbeck *et al* is also included in this issue of *WJG*.

Inhibitory receptors: PD-1

The inhibitory receptor PD-1 ("programmed cell death 1") has been demonstrated to be a strong marker for exhausted virus-specific CD8+ T cells in the LCMV mouse model. The antibody-mediated blockade of the interaction between PD-1 and its ligand PD-L1 led to the restoration of cytokine secretion, proliferation, and cytotoxicity by the exhausted virus-specific CD8+ T cells and a substantial reduction in viral load[57]. Similar roles of PD-1 have been shown in human chronic viral infections[58] such as HIV[59], HBV[60,61], and HCV. In the acute phase of HCV infection, similar to LCMV infection, PD-1 is up-regulated on HCV-specific CD8+ T cells independent of outcome. However, in individuals with resolving infection PD-1 expression decreases soon, while in patients with a chronic course of infection, HCV-specific CD8+ T cells remain PD-1 positive[62]. This finding is in parallel with

the "stunned" phenotype of HCV-specific CD8+ T cells in the early acute phase of infection, which is restored in resolving infection but remains in persisting infection[2,3,24]. In chronic HCV infection, HCV-specific CD8+ T cells in the peripheral blood[63] as well as liver[64] have been shown to express high levels of PD-1. Blockade of PD-1/ PD-L1 interaction by antibodies restored cytokine production and proliferation of the exhausted CD8+ T cells from acute and chronic infection *in vitro*.

It is important to note, however, that the antibody-mediated blockade of the PD-1/PD-L1 pathway in chronically LCMV-infected mice did not result in viral clearance although a significant reduction of viral load was achieved. Even more importantly, PD-L1-/- mice died due to immunopathologic damage after infection with a LCMV strain usually establishing persistent infection[57]. These findings indicate that a subtle balance in the blockade of the PD-1/PD-L1 pathway must be granted before it can be applied in the clinics.

Inhibitory cytokines: IL-10

Two recent reports on the role of IL-10 in the dysfunction of virus-specific T cells and viral persistence gained much attention in the field. These reports showed that in mice with persistent LCMV infection, IL-10 was highly up-regulated early in infection, which was associated with the dysfunction of virus-specific CD4+ and CD8+ T cells. The blockade of the IL-10/IL-10 receptor (IL-10R) pathway by a genetic approach or by an anti-IL-10R antibody early in infection, however, led to the restoration of T cell function and to clearance of infection[65-67].

Although these reports definitely point towards an important general mechanism of T cell dysfunction, a role of IL-10 in HCV infection has been postulated before, and IL-10 therapy has even been tested in clinical trials in HCV infected patients. Indeed, many reports showed an association of IL-10 polymorphisms and outcome, disease progression, or response to antiviral therapy of HCV infection[68-74], while other studies failed to confirm these data[75-79]. Clinical trials with administration of recombinant IL-10 to patients with chronic HCV infection who had failed antiviral therapy with interferon-alpha led to a decrease in transaminases and histological disease progression; however, viral titers strongly increased in some IL-10 treated patients[80,81]. This indicates that IL-10 might not only mediate viral dysfunction and thus facilitate viral persistence in acute infection, but may also reduce immunopathology in the chronic phase of infection. In this context, it is important to point out, however, that IL-10/ IL-10R blockade in the LCMV mouse model did not result in severe immunopathology[65,66].

The exact mechanism of HCV-induced up-regulation of IL-10 remains elusive. Some groups have reported induction of IL-10 production by monocytes[82] or natural killer (NK) cells[83] through core[74,84], non-structural protein 3[84], or 4[82]. More intriguingly, HCV-specific CD8+ T cells with regulatory properties which produce IL-10 have been described in the liver of chronically HCV infected patients[54]. These IL-10 producing intrahepatic CD8+ T cells were associated with mild inflammation and low progression of fibrosis in liver histology[55], once more suggesting that IL-10 may protect from immunopathology in chronic HCV infection. Blockade of the IL-10 pathway by anti-IL10R antibodies *in vitro* led to increased HCV-specific CD4+ T cell responses[85]. In addition, antiviral therapy led to reduced production of IL-10 by virus-specific T cells in patients with chronic HCV infection[86]. A direct inhibition of the IL-10 pathway, however, needs further careful evaluation in additional animal models before it can be transferred to men.

Viral escape

HCV is a RNA virus with an enormous replication rate (approximately 10^{12} virions per day) with a RNA-dependent RNA polymerase that lacks a proofreading function. Therefore, multiple viral variants, called quasispecies, circulate in a single individual. It has been suggested that the selection of viral variants escaping from CD8+ T cell responses might facilitate the persistence of HCV infection. Indeed, the first evidence for viral escape in HCV infection came from chronically infected patients[87] and experimentally infected chimpanzees[88,89]. Chronically infected patients harbored variant viral sequences in targeted epitopes which were non-immunogenic and not cross-reactive with the prototype peptides. These viral escape mutations remained fixed over a follow-up time of up to four years, indicating that escape mutations occur early in infection[87]. In the chimpanzee model, it could further be demonstrated that viral escape mutations occurred during the first 16 wk of infection and were associated with a chronic course of infection[89].

Important additional information came from studies in acutely infected patients[90-92] as well as population-based approaches[90,93]. In these studies, viral escape from CD8+ T cell responses was demonstrated in patients developing persistent infection[90-92], but not in individuals with resolving infection[91,92]. Interestingly, many mutations outside of targeted CD8+ T cell epitopes represented conversion to consensus[91], and the transmission of an HLA-B8 associated escape mutation to an HLA-B8 negative subject resulted in rapid reversion of the mutation[90]. These results were supported by a study in a well-defined cohort of Irish women accidentally infected with HCV from a single source more than 20 years ago. In this unique cohort, amino acid substitutions in known epitopes were directed away from consensus in women having the HLA allele associated with that epitope, and toward consensus in those lacking the allele[93]. These findings are in agreement with the concept of viral fitness cost, indicating that viral escape mutations are often associated with a reduced replicative capacity of the virus[94]. In the absence of the T cell pressure, e.g. upon transmission to an individual negative for the restricting HLA allele, the virus reverts to consensus and thus regains its full replicative capacity. This phenomenon has been analyzed in more detail in the background of an immunodominant HLA-A2 restricted epitope, identifying that certain amino acid residue substitutions abolish HLA binding without strongly influencing viral replication, while some substitutions lead to a strong reduction of viral fitness[95]. Importantly, there might be some CD8+ T cell epitopes which are not affected by viral escape

due to high functional constraints. For example, we have recently identified an HLA-A26 restricted epitope located at the NS5A/5B cleavage site which was targeted in all studied HLA-A26+ patients (3/3) with acute HCV infection and a significant number of patients with chronic HCV infection (3/15). However, the epitope sequence was highly conserved in HLA-A26 positive and negative patients, indicating that viral escape did not occur in this functionally constrained region[96].

Based on the finding that immunodominant CD8+ T cell epitopes leave their footprint in viral sequences in chronic HCV infection[90], viral genome sequencing studies were performed in order to identify footprints of additional potential CD8+ T cell epitopes[97,98]. In addition to previously defined epitopes, these studies identified HLA allele dependent polymorphisms and thus candidate CD8+ T cell epitopes. Importantly, the strongest association with any HLA allele in the study by Timm *et al* was found for HLA-B27 in a region that was shown to contain an immunodominant HLA-B27 restricted CD8+ T cell epitope by an independent study in another patient cohort[99].

There are different molecular mechanisms by which a certain mutation escapes from the CD8+ T cell response. Especially those mutations located at the HLA binding anchors, usually P2 and the C-terminal amino acid, lead to the interruption of the peptide binding to the HLA molecule. Mutations in the center of the epitope, in contrast, are more likely to interfere with T cell receptor (TCR) recognition[95]. Mutations in the flanking region, however, prevent proteasomal epitope processing[90,100,101].

The determinants of viral escape are less understood. In the chimpanzee model of HCV, it has been shown that upon depletion of CD4+ T cells in the acute phase of infection viral escape from the CD8+ T cell response occurs and is associated with a persistent course of infection[46]. This finding has led to the hypothesis that viral escape is caused by insufficient CD4+ help. Other studies indicate that a limited T cell receptor (TCR) diversity might be responsible for viral escape[102]. Of note, viral escape does not occur in the context of dysfunctional CD8+ T cell responses[103]. The strong association between HLA-B27 and viral escape within an immunodominant HLA-B27 restricted epitope as well as the suggestion that escape variant epitopes might preferentially be restricted by HLA-B alleles indicates that the restricting HLA allele background also plays an important role in determining viral escape[97-99].

Lack of homing to the liver

Experimentally HCV infected chimpanzees which progressed to viral persistence without temporary viral control lacked virus-specific CD8+ T cell responses in the liver despite of detectable responses in the peripheral blood[2]. This finding led to the tempting hypothesis that the failure of the virus-specific CD8+ T cell response might be caused by an insufficient homing to the primary location of infection, the liver. However, in chronically HCV infected patients virus-specific CD8+ T cells are detectable and even enriched in the liver[22,25,104-108]. In a comprehensive study comparing the overall breath and vigor of CD8+ T cell responses in the peripheral blood and liver of chronically HCV infected patient's, we found that virus-specific CD8+ T cell responses were strongly enriched in the liver. Many responses were only detectable in the liver; however, few responses were limited to the peripheral blood (Neumann-Haefelin *et al*, unpublished results). Therefore, it is possible that a defective homing of HCV-specific CD8+ T cells or their rapid deletion in the liver also contributes to T cell failure and viral persistence in a subset of patients.

ROLE OF THE HOST HLA CLASS I BACKGROUND

CD8+ T cells recognize antigens presented by human leukocyte antigen (HLA) class I molecules. It has, therefore, been suggested that different HLA class I alleles are associated with differential outcomes of HCV infection, e.g. viral clearance versus persistence[109]. Analysis of the role of HLA alleles in viral infections are hindered by multiple factors, including the wide polymorphism of HLA alleles, their association with other genetic characteristics e.g. in certain racial backgrounds (founder effect)[110], and the variability of viral strains (genotypes, quasispecies *etc*.). However, an Irish cohort of women accidentally infected with HCV (genotype 1b) from a single source more then 20 years ago, represents a homogeneous group in which the role of HLA alleles in the outcome of HCV infection could be studied[111]. Importantly, the HLA class I alleles A3, B27 and Cw*01 were significantly associated with viral clearance, while B8 was associated with viral persistence. Interestingly, the strongest protective effect was observed for HLA-B27: 80% (12/15) of B27 positive women were able to clear the infection spontaneously, while only a minority developed chronic infection. We recently identified an immunodominant HLA-B27 restricted HCV-specific CD8+ T cell epitope, which was targeted in the vast majority (5/6) of B27 positive Irish women who had cleared the infection[99]. Of note, such a clear dominance of a single epitope-specific CD8+ T cell response has not been described for any other HLA allele in HCV infection. In chronically infected patients, still a remarkable percentage of patients (3/8) recognized the epitope. However, most B27 positive chronically infected patients had evidence of viral escape within the otherwise conserved viral region containing this epitope. Thus, a single immunodominant HLA-B27 restricted CD8+ T cell epitopes might mediate both, clearance of HCV infection in the majority of B27 positive individuals, and viral evolution associated with viral persistence in a minority of individuals.

Strikingly, a very similar frequency of viral escape variation was demonstrated within an immunodominant HLA-B8 restricted CD8+ T cell epitopes[90]. This indicates that in the background of both, a protective HLA allele (B27) as well as a detrimental HLA allele (B8) the principle mechanisms of CD8+ T cell failure might be the same. More precise details such as viral fitness cost associated with the respective escape variation[94,95], T cell receptor (TCR) diversity[102] or heterologous immunity[112,113]

may play an additional critical role in the definition of a protective HLA allele.

Two other population studies in more heterogeneous cohorts showed an association between HLA-B57 and HCV clearance in Caucasian as well as African Americans and West Africans[114,115]. Interestingly, HLA-B27 and HLA-B57 have also been shown to be protective in HIV infection, being strongly associated with low viral titers, low CD4+ T cell decline, and long-term non-progression of the disease[116]. Thus, a picture emerges that the same HLA alleles may confer protection in different clinical infections, indicating that similar mechanisms of viral control and disease progression apply in these infections. A better understanding of the host-virus interactions leading to different clinical outcomes of HCV infection will be important not only to understand the mechanisms of viral clearance and persistence, but also for the development of new antiviral vaccine strategies.

CONCLUSION

CD8+ T cells are generally thought to be the major effectors in viral infections; however, multiple host and viral mechanisms contribute to the failure of antiviral CD8+ T cell responses and viral persistence in the majority of HCV infected patients. In the last few years compelling progress has been achieved in the understanding of these mechanisms (compare with[117]). These findings are not only important for the development of successful immunoprophylactic approaches, but may also be more directly adopted for immunotherapeutic interventions.

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