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Review

New insights into the immunopathogenesis of chronic hepatitis C

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ABSTRACT

Despite the high propensity of hepatitis C virus to establish chronic viral persistence, immune-mediated viral clearance occurs in some patients, fostering hopes that therapeutic induction of specific antiviral immune responses might be able to contribute to viral clearance in chronically infected patients. Indeed, recent clinical trials of therapeutic vaccination have provided clear proof of concept that specific immunotherapy can reduce the viral load in some patients. Further improvement of these strategies will depend on a detailed analysis of the immunopathogenesis of chronic hepatitis C. Recent advances in our understanding of the mechanisms of down-regulation of virus-specific immune responses during chronic infection, including the role of regulatory T cells and inhibitory molecules such as programmed death receptor 1, may open up new avenues for second-generation immunotherapeutic interventions.

Contents

1. Introduction

Hepatitis C is one of the most common chronic viral infections in humans, affecting about 170 million individuals worldwide (Lauer and Walker, 2001). The major virological, epidemiological, clinical and immunological features of the disease are summarized in Table 1. Worldwide, chronic hepatitis C is a major cause of cirrhosis and hepatocellular carcinoma, and in Western countries it is a leading indication for liver transplantation. Even following liver transplantation, graft re-infection with hepatitis C virus is ubiquitous. Today we are facing an increasing number of patients with hepatitis C virus-associated liver cirrhosis in transplant patients (Gane, 2008).

The currently available treatment for hepatitis C consists of pegylated interferon-alpha in combination with ribavirin which leads to a sustained virological response in about 50% of patients who can tolerate the treatment (Hoofnagle and Seeff, 2006). New antiviral drugs, such as inhibitors of the viral protease and polymerase, are in clinical development, but if given as monotherapy, viral resistance develops very rapidly. All these new drugs are therefore currently administered in combination with PEG-interferon and ribavirin (Sarrazin et al., 2007). Recent results from phase II clinical trials suggest that the sustained virological response in genotype 1 patients can be increased to about 60–70% (Lang and Weiner, 2008; Stauber and Kessler, 2008). However, this still leaves a substantial number of patients who cannot be cured with currently available treatments, and a large proportion of patients with liver cirrhosis who cannot tolerate interferon-based regimens.

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Table 1
Basic features of hepatitis C virus infection.

Virus classification and structure	Hepatitis C virus (HCV) is the only member of the genus *Hepacivirus* in the family *Flaviviridae*. The 9.6 kb single-stranded, positive-sense RNA genome encodes a single open reading frame flanked by 5′ and 3′ untranslated regions. There are 7 major HCV genotypes and so far 84 subtypes; in the US, Europe, and Australia genotypes 1a or 1b are most prevalent followed by genotypes 2 and 3. In contrast the majority of Japanese patients are infected by genotype 1b, followed by genotype 2. Some genotypes predominantly occur in locally restricted areas such as genotype 4 in Egypt, genotype 5 in South Africa, and genotype 6 in East Asia.
Epidemiology	HCV infects only humans and chimpanzees. There are no approved vaccines. Today the virus is transmitted mainly though injecting drug use or other types of direct blood contact; sexual and perinatal transmission are rare. Nearly 200 million people are chronically infected worldwide. In the United States and Europe, there are estimated to be some 3.2 million and 5 million infected persons, respectively. Rates of HCV infection are higher among people infected with HIV.
Clinical course	Acute hepatitis C is frequently asymptomatic or causes a mild to moderate disease, fulminant acute hepatitis C is extremely rare. Some 15–25% of patients are able to eliminate the virus without treatment. Chronic HCV infection is diagnosed on the basis of anti-HCV antibodies, PCR testing for circulating virus and the detection of abnormal liver function. The CDC estimates that for each 100 people with HCV, 75–80 will become chronically infected, 60–70 will develop liver disease, 5–20 will develop cirrhosis over a period of 20–30 years, and 1–5 will die of the consequences of viral infection.
Antiviral therapy	Current therapy consists of a combination of pegylated interferon-alpha and ribavirin for 24–48 weeks, depending on genotype and viral load. Patients with genotypes 2 and 3 have a 70–80% sustained virological response rate (HCV RNA undetectable in serum for >6 months after the end of treatment) while the rate is 40–45% for genotype 1.
Host response to hepatitis C	HCV interferes at several levels with the activation of innate immune responses, in particular with signalling pathways leading to the secretion of type I interferons, which may also affect the successful induction of specific immune responses. Nevertheless, in the absence of immunosuppression, patients infected with HCV regularly develop antibodies against several viral antigens, including the envelope proteins. The development of virus-specific antibodies, however, does not correlate with control of viremia or the outcome of acute hepatitis C. Strong and maintained virus-specific CD4+ and CD8+ T cell responses are thought to be required for spontaneous viral clearance and can be detected in resolved patients for more than 20 years after successful elimination of HCV.

A promising new approach for the treatment of chronic hepatitis C may be therapeutic vaccination, which aims at the induction of virus-specific T cell responses for viral clearance (Lang and Weiner, 2008). The rational development of a therapeutic vaccine, however, depends on a detailed understanding of both the correlates of immunity and the pathogenesis of chronic viral persistence. Both aspects of virus–host interaction can be well studied in acute hepatitis C, in which a self-limited course of disease occurs in about 15–30% of patients (Gerlach et al., 2003). In this unique patient cohort, the correlates of a successful antiviral immune response have been extensively studied in comparison to the evolution of the virus-specific immune response in patients developing chronic hepatitis C (Chang et al., 1999; Cucchiarini et al., 2000; Diepolder et al., 1995; Gerlach et al., 1999; Gruener et al., 2000; Kaplan et al., 2008; Lamonaca et al., 1999; Lechner et al., 2000a,b; Lucas et al., 2007; Missale et al., 1996; Shoukry et al., 2004; Thimme et al., 2001). In addition, new immunological tools such as HLA class I and class II tetramers allow the characterization of even dysfunctional HCV-specific T cells in patients with chronic hepatitis C and thus offer the opportunity to monitor virus-specific T cell responses during immunotherapy in correlation with viral kinetics (Day et al., 2003; Lechner et al., 2000a,b; Lucas et al., 2007; Radziewicz et al., 2008b; Ulsenheimer et al., 2006). This review summarizes the current perception of successful and failing HCV-specific T cell responses and how this knowledge may be translated into successful antiviral immunotherapies.

2. Immunological mechanisms of spontaneous viral clearance

About 15–30% of patients with acute hepatitis C achieve spontaneous viral clearance (Lauer and Walker, 2001). This observation is exciting for two reasons: first, it demonstrates that the patient's immune response can clear HCV, and second, understanding in detail how this is accomplished may aid the development of successful immunotherapies for patients with chronic hepatitis C. The importance of IFN-γ producing HCV-specific CD8+ T cells was shown both in patients with acute hepatitis C who achieved spontaneous viral clearance and in experimental infection in chimpanzees (Gruener et al., 2000; Shoukry et al., 2003; Thimme et al., 2001). In an interesting cohort of patients who acquired HCV following a needle stick injury and were followed throughout the incubation period until the onset of clinical hepatitis, the development of the HCV-specific CD8+ T cell response could be studied from the beginning: while virus-specific CD8+ T cells could already be detected by HLA class I tetramers during the incubation period, a decline in viral load was only observed when these specific CD8+ T cells acquired the ability to secrete IFN-γ (Thimme et al., 2001). These data are supported by several studies in the chimpanzee model. Most convincingly, it could be shown that depletion of CD8+ T cells prevents viral elimination, and viral eradication was observed only upon recovery of IFN-γ-producing CD8+ T cells (Bowen and Walker, 2005; Shoukry et al., 2003).

Given the importance of HCV-specific T cells for viral elimination and the high genetic variability of HCV, it did not come as a surprise that viral escape mutations occur and that they may represent an important mechanism for the failure of the immune response to eradicate the virus in the majority of patients (Bowen and Walker, 2005). As seen with HIV, the viral sequence adapts to the patient's HLA class I background. Associations of certain mutations with HLA class I alleles have been demonstrated (Ray et al., 2005). Nevertheless, when potential epitopes in a given patient are sequenced, escape mutations do not occur universally, strongly pointing towards other mechanisms of down-regulation of virus-specific CD8+ T cells, as will be discussed below.

Antigen-specific CD4+ T cells play an important role in the activation of antigen-presenting cells and in the induction and maintenance of an antigen-specific CD8+ T cell response. In the case of hepatitis C, two lines of evidence support an important role of HCV-specific CD4+ T cells. First, strong and sustained HCV-specific CD4+ T cell responses have consistently been associated with spontaneous viral clearance (Diepolder et al., 1997; Takaki et al., 2000). These responses seem to be stronger against the non-structural proteins, and they can be detected for more than 20 years following successful viral clearance. Interestingly, a subset of patients with acute hepatitis C only transiently controls HCV replication with very low or undetectable viremia for a period of weeks or a few months (Gerlach et al., 1999). During the phase of viral control, these patients exhibit HCV-specific CD4+ T cell responses indistinguishable from those of patients who have achieved permanent

viral clearance. Upon viral recrudescence, however, HCV-specific CD4+ T cells become dysfunctional and are subsequently lost (Lucas et al., 2007; Ulsenheimer et al., 2006). It is unclear whether CD4+ T cell dysfunction always precedes viral recrudescence or whether it occurs as a consequence of recurrent viremia. The second line of evidence for an important role of HCV-specific CD4+ T cells also comes from experimental infection of chimpanzees, in which depletion of CD4+ T cells led to chronic viremia despite the presence of functional HCV-specific CD8+ T cells (Grakoui et al., 2003). Interestingly, CD4+ T cell depletion seemed to facilitate viral escape mutations within CD8+ T cell epitopes.

In conclusion, the coordinate interplay between functional virus-specific CD4+ and CD8+ T cells seems to be required to achieve permanent viral clearance. While some patients are able to mount such a response and to maintain it long-term, the majority of individuals with acute hepatitis C fail to permanently clear the virus because of a combination of mechanisms, including viral escape mutations and T cell exhaustion. As will be discussed below, the latter has recently drawn much attention, due to the identification of inhibitory molecules that are amenable to therapeutic intervention.

3. Immunopathogenesis of chronic viral persistence

3.1. HCV and the adaptive immune response

Despite the multitude of interactions between HCV and different aspects of the innate immune response, virus-specific CD4+ and CD8+ T cells and antibody responses are induced in virtually every immunocompetent patient with acute hepatitis C. In fact, several studies have not found a significant difference in the first weeks of acute hepatitis C between patients who subsequently clear the virus and those who develop chronic infection (Cox et al., 2005a). Moreover, a substantial portion of patients with chronic viral persistence go through a phase of incomplete viral control with viral titres below 10^4 IU/ml, which is 2–3 log lower than average titres in chronic hepatitis C (Gerlach et al., 1999). The eventual loss of this partial control is accompanied by a sharp decline in HCV-specific CD4+ T cell responses. Using HLA class II tetramers for the direct ex vivo staining of HCV-specific CD4+ T cells, it was demonstrated that loss of function, i.e. secretion of IFN-γ and proliferation, preceded the physical deletion of HCV-specific CD4+ T cells (Lucas et al., 2007). In contrast, patients with self-limited acute hepatitis C maintained strong CD4+ T cells for many years following resolution of disease (Takaki et al., 2000).

HCV-specific CD8+ T cell responses that are associated with spontaneous viral clearance tend to be multispecific and polyclonal (Cox et al., 2005a; Lauer et al., 2002, 2004, 2005). Because of the high variability of HCV, escape mutations in CD8+ T cell epitopes are common, and they are expected to play a major role in chronic viral persistence (Cox et al., 2005b; Ray et al., 2005; Timm et al., 2004). Many mutations, however, come at a cost of viral fitness, and in some epitopes mutations are only possible at certain positions. In the case of an immunodominant HLA-B27-restricted CD8+ T cell epitope, it has been shown convincingly that mutations at HLA binding positions that would lead to a loss of HLA binding were not tolerated in the replicon system. Mutations at amino acid positions that were presumed to interact with the T cell receptor did not severely affect replication, but more than one mutation were necessary to achieve immune escape (Neumann-Haefelin et al., 2006, 2008). In fact, the same authors have shown that most chronically infected HLA-B27 positive patients harbour viruses with multiple mutations in this epitope, strictly sparing the HLA binding sites (Neumann-Haefelin et al., 2006). This strongly emphasizes the importance of T cell mediated immune pressure on the virus. In contrast, when HCV genomes of chronically infected patients are analyzed with regard to potential CD8+ T cell epitopes, most epitopes are conserved, which suggests other, most likely immunomodulatory, mechanisms of "viral escape" (Ray et al., 2005). In fact, functional impairment of HCV-specific CD8+ T cells was demonstrated some time ago (Lechner et al., 2000a; Wedemeyer et al., 2002), but very recent advances in the field of immunoregulatory receptors on T cells, most notably programmed death receptor 1 (PD-1), have expanded our understanding of T cell regulation (Barber et al., 2006). Together with new insights into the role of regulatory T cells in chronic viral infections, this will lead to new opportunities to manipulate virus-specific T cell responses in vivo.

3.2. The role of regulatory T cells in acute and chronic hepatitis C

In 1995, a new type of T cell with a CD4+CD25++ phenotype was identified that was able to suppress immune responses (Takahashi et al., 1998). These cells, called "regulatory T cells" (Treg), were shown to be crucial for the maintenance of self-tolerance, as their depletion in mice led to a severe autoimmune syndrome. Later in 2003, the transcription factor FOXP3 was shown to be the main inducer of a Treg phenotype (Hori et al., 2003). The expression of FOXP3 is a highly specific marker of regulatory T cells in mice, but is less specific in humans, since it is also readily induced by T cell activation (Roncarolo and Gregori, 2008). Today the FOXP3+CD25+CD4+ regulatory T cells are divided into two groups: the so-called natural Tregs are supposed to be thymus-derived and play a major role in the prevention of autoimmunity (Sakaguchi, 2008). Suppression of T cell effector function is thought to be mediated by cytokines like IL-10 or TGF-β and cell-to-cell contact (Vignali, 2008). The frequency in the peripheral blood ranges from 2 to 5% of CD4+ T cells but can be considerably higher in inflamed tissues.

The other Treg subtype is thought to be derived from antigen-specific effector T cells and has been shown to occur during infectious diseases, autoimmune disease and in malignancy. These peripheral Tregs may be involved in the down-regulation of specific immune responses. In the case of infectious diseases, there is evidence that the appearance of Treg represents a physiological mechanism to terminate a successful immune response (Robertson and Hasenkrug, 2006; Rouse et al., 2006; Rouse and Suvas, 2004; Suvas and Rouse, 2006). In this scenario, Treg may limit immune-mediated tissue damage after an infection has been controlled. On the other hand, premature induction of antigen-specific Treg could well be a contributing factor in the evolution of chronic infections such as viral hepatitis B and C or HIV.

Several studies have also addressed the role of Treg in chronic hepatitis C in comparison to hepatitis B and HIV. In both hepatitis B and C, it has been shown that depletion of CD25++ CD4+ Treg in vitro increased virus-specific CD4+ and CD8+ T cell responses, whereas the addition of purified Treg in cell culture suppressed T cell effector mechanisms in a dose-dependent manner (Boettler et al., 2005; Chang, 2007; Ebinuma et al., 2008; Kinter et al., 2007a,b; Li et al., 2007; Manigold and Racanelli, 2007; Sprengers et al., 2007; Stoop et al., 2007). Importantly, however, this effect of Tregs was not only observed for HCV-specific T cell responses, but also for responses against control antigens such as influenza (Franzese et al., 2005). Studies in the only animal model of HCV infection, the chimpanzee, have revealed that both the frequency and the degree of suppression of bulk Tregs did not differ between recovered chimpanzees and chimpanzees with chronic hepatitis C viral persistence (Manigold et al., 2006). In humans, however, several studies have confirmed that the overall frequency of CD25++ CD4+ Treg and also FOXP3+ CD4+ Treg is increased in chronic hepatitis C (Cabrera et al., 2004; Ebinuma et al., 2008; Smyk-Pearson et al., 2008). In acute hepatitis C an increased frequency and increased

suppressor activity of Treg was also observed, which was independent of the clinical outcome of disease (Smyk-Pearson et al., 2008). However, in those patients who recovered from acute hepatitis C, Treg suppressor activity declined following viral clearance.

One of the most critical questions is obviously whether these Treg are HCV-specific or whether the expansion of Tregs is caused by some other factor produced during virus–host interaction. One study has used purified CD25+CD4+ cells which were subsequently stimulated by HCV antigen (Bolacchi et al., 2006). Based on the observed HCV antigen-specific IL-10 secretion, the authors concluded that HCV antigen-specific Tregs exist. However, by sorting CD25+CD4+ T cells, the authors could not exclude that they had activated effector cells within the population that were responsible for the HCV-specific IL-10 secretion. Very recently, Ebinuma et al. demonstrated FOXP3 expression in HCV-specific CD4+ T cells following short-term in vitro expansion, and also directly ex vivo in two patients with chronic hepatitis C using an HCV-specific HLA class II tetramer (Ebinuma et al., 2008). The authors also found a high rate of FOXP3 expression in HCV antigen-specific T cell lines, which was further enhanced by the addition of TGF-β. Although it has been shown that these culture conditions can induce FOXP3 expression in human CD25− CD4+ effector T cells in vitro, there is evidence that a similar cytokine milieu may be present in the liver of patients with chronic hepatitis C, which could favour the local transformation of effector T cells into regulatory T cells.

Our group has addressed the issue of antigen-specific Tregs in a population of acute hepatitis C patients using direct ex vivo staining with HLA class II tetramers and FOXP3. In this study, FOXP3 expression in HCV-specific CD4+ T cells was very low (<2%) in most cases, but short-term increases of FOXP3 expression up to 30% of specific CD4+ T cells were observed in three of ten patients towards the end of the acute illness (Heeg et al., submitted for publication). These time points were also associated with a transient loss of CD4+ T cell effector function and in two of three cases with transient viral recrudescence. Importantly, however, no increase of FOXP3 expression was observed at time points associated with evolution to chronic viral persistence, indicating that HCV-specific FOXP3+ CD4+ T cells are not a major mechanism for the induction of chronic viral persistence.

Based on these studies, it seems likely that the expansion of Tregs that is observed in chronic hepatitis C is rather due to HCV non-specific Tregs. In this context, a recent paper by Dolganiuc et al. may be revealing, as the authors showed that myeloid dendritic cells derived from chronic hepatitis C patients are able to stimulate proliferation of regulatory T cells which inhibit proliferation of HCV-specific T lymphocytes (Dolganiuc et al., 2008). In addition to these studies, which mainly focused on peripheral blood, some investigations have addressed the frequency of Treg in liver biopsies. Although the question of antigen specificity could not be addressed, a considerable accumulation of FOXP3 expressing CD4+ T cells has been observed in the livers of patients with chronic hepatitis C (Ward et al., 2007). In conclusion, despite the unequivocal proof of the occasional expression of FOXP3 in HCV-specific CD4+ T cells, it is unclear whether HCV-specific Treg play a major role in the pathogenesis of chronic viral persistence. Nevertheless, an increased frequency of Treg is observed in the peripheral blood of patients with chronic hepatitis C, and Treg also seem to accumulate in the inflamed liver. Recent evidence indicates that the interaction of HCV with parts of the innate immune system (for example, myeloid dendritic cells) can lead to the expansion of Treg, which may facility the suppression of HCV-specific CD4+ and CD8+ T cells in the liver.

In addition to classical FOXP3+ CD4+ regulatory T cells, IL-10-producing Tr1 cells, TGF-β-producing Th3 and CD8+ regulatory T cells have been described, and there is evidence that increased production of IL-10 (Kaplan et al., 2008) and TGF-β (Alatrakchi et al., 2007) play a role in the pathogenesis of chronic viral persistence. Blocking any of these cytokines for the restoration of T cell function could be an additional or complementary approach to blocking inhibitory receptors such as PD-1 or CTLA-4, which are discussed below.

3.3. The role of PD-1 in the down-regulation of HCV-specific T cell responses

Programmed death receptor 1 has recently been described as a major mediator of CD8+ T cell exhaustion (Barber et al., 2006). In a mouse model of LCMV infection, it was convincingly shown that virus-specific CD8+ T cells expressed high levels of PD-1 during chronic infection, and that treatment with anti-PD-L1 antibodies could restore the function of virus-specific CD8+ T cells, leading to a decrease in viral load. The role of PD-1 in chronic viral infection in humans has subsequently been studied in detail. In both HIV and hepatitis C virus infection, PD-1 expression on virus-specific CD8+ T cells could be confirmed (Bowen et al., 2008; Day et al., 2006; Golden-Mason et al., 2007; Kasprowicz et al., 2008; Maier et al., 2007; Penna et al., 2007; Radziewicz et al., 2007; Urbani et al., 2006). In vitro studies using antigen-specific stimulation in the presence of PD-1 antibodies or antibodies against the ligand PD-L1 and PD-L2 also led to restoration of IFN-γ production by virus-specific CD8+ T cells.

When the evolution of PD-1 expression was studied in acute hepatitis C, it was found that PD-1 expression was high on virus-specific CD8+ T cells during the acute phase of HCV infection, irrespective of the outcome. However, in patients who achieved spontaneous viral clearance, PD-1 expression tended to decline or to disappear on virus-specific CD8+ T cells, whereas in patients who developed chronic infection, PD-1 expression persisted at high levels. A correlation was established between the strength of PD-1 expression and viral load (Kasprowicz et al., 2008). Interestingly, intrahepatic HCV-specific CD8+ T cells seem to express higher levels of PD-1, compared to their counterparts in the peripheral blood, and blocking PD-1 is not sufficient to restore effector function to these intrahepatic cells (Nakamoto et al., 2008; Radziewicz et al., 2008a).

Whereas the importance of PD-1 for CD8+ T cell exhaustion in hepatitis C is now well documented, much less is known about PD-1 expression on HCV-specific CD4+ T cells. In preliminary experiments, our group found that PD-1 expression on HCV-specific CD4+ T cells paralleled the kinetics of PD-1 expression on CD8+ T cells, both in acute and chronic hepatitis C (Heeg et al., unpublished observation). In a recent paper, PD-1 blockade was shown to restore HCV-specific CD4+ T cell function and proliferation (Urbani et al., 2008). These observations offer a great opportunity to manipulate virus-specific immune responses in vivo, by treating patients with antibodies to PD-1. Clinical trials using PD-1 antibodies have been started for a number of indications including hepatitis C (http://www.news-medical.net/?id=43340). Whereas some safety data are already available from studies in malignancy, no results from the HCV trial have been reported so far.

From studies of HIV infection, it appears possible that other immunoregulatory molecules such as CTLA-4 are also involved in the down-regulation of virus-specific T cell responses (Kaufmann et al., 2007). Data from HIV-infected patients also indicate that the pattern of PD-1 and CTLA-4 expression may vary between different individuals, indicating that different modes of immunoregulation may be active in different subsets of patients or in different phases of chronic infection. There is certainly a need for more detailed investigation to define the relative contribution of different immunoregulatory pathways in chronic hepatitis C. Nevertheless, the identification of this dominant pathway, combined with the possibility of interfering with the PD-1 pathway in vivo, offers

great hope for the further development of immunotherapies to treat chronic hepatitis C.

4. How can therapeutic vaccines overcome T cell nonresponsiveness?

During chronic infection, viral antigen is abundant in the body, and virus-specific T cells are continuously exposed to their specific antigen. T cell non-reactivity is assumed to result from antigen presentation by non-professional antigen-presenting cells or from a lack of activation of professional antigen-presenting cells. That is where therapeutic vaccines could make the difference: by targeting professional antigen-presenting cells such as dendritic cells, and by providing enough "danger" signals to ensure their activation. The unresolved questions are to what extent exhausted T cells can be revived through optimum antigen presentation, and whether concomitant blockade of inhibitory receptors such as PD-1 is required to restore T cell function. While the simultaneous blockade of PD-1 or immunosuppressive cytokines such as IL-10 has been shown to increase the potency of vaccination in animal models, this remains to be proven in human diseases.

5. Current strategies of immunotherapy of chronic hepatitis C

Immunotherapy for hepatitis C virus infection has only recently reached the clinical phase of development. The first therapeutic vaccination was performed with a number of HCV-derived CD4+ and CD8+ T cell epitopes adjuvanted with poly-arginine in patients who had failed to interferon treatment (Klade et al., 2008). Although the vaccination was very well tolerated, effects on viral load were detectable in only a few individuals. Nevertheless, there was evidence of a correlation between vaccine-induced immune responses and a decrease in viral load in some cases. When the same candidate vaccine was administered to treatment-naïve patients and the adjuvant was augmented by a topical TLR7 agonist, a small but significant decrease in the viral load was observed for the entire cohort (http://www.intercell.com/main/forbeginners/news/not-in-menu/news-full/back_to/hepatitis-c-virus-vaccine/article/favourable-six-months-follow-up-results-from-intercells-phase-ii-therapeutic-hepatitis-c-program/).

Because a major limitation of this approach is the small number of epitopes, two other studies have used larger parts of the HCV genome to cover more epitopes and more HLA-restrictions. A recombinant MVA (Modified Vaccinia Virus-Ankara) virus containing the HCV NS3-NS4-NS5B genes was given three times to patients with chronic hepatitis C who had failed a previous interferon-based therapy. In 6 of 15 patients, a decline in the viral load of >0.5 log was observed, and the virological response was again correlated with the induction of virus-specific T cell responses as measured by IFN-γ Elispot (http://www.transgene.fr/us/pdf/communique_presse/communiques_divers_2008/PR-US-TG4040-19-05-2008.pdf). A Swedish company has developed a DNA vaccine containing HCV-NS3-4A which was administered by in vivo electroporation in treatment-naïve patients with chronic hepatitis C. Four of six patients in the higher dose group showed a viral load decline >0.5 log (http://www.cisionwire.com/tripep/tripep-s-chronvac-c–treatment-shows-antiviral-effect-in-patients-with-chronic-hepatitis-c-also-in-the-high-dose). In both of these studies, transient viral load reductions as great as 1–2 log were observed and could be correlated with HCV-specific T cell responses.

These studies provide first proof of concept that therapeutic immunization has a potential in the treatment of chronic hepatitis C. Notably, in all studies, therapeutic vaccination was very well tolerated by the patients and so far there has been no evidence of the induction of significant or even fulminant hepatitis. Although it is still unlikely that a larger fraction of patients will be completely cured by immunotherapeutic approaches at their current state, a better understanding of the immunopathogenesis of chronic hepatitis C and the virus-specific immune response nevertheless offers a unique opportunity to further improve specific immunotherapy.

6. Promising directions for future research

While the role of HCV-specific regulatory T cells still needs to be defined more precisely, immunoregulatory molecules such as PD-1, CTLA-4 and others have a clear potential to explain T cell dysfunction in chronic hepatitis C. However, the fact that these inhibitory receptors are expressed in virus-specific T cells in chronic infection does not explain why some patients spontaneously clear the infection while others do not. Future research combining studies of innate and adaptive immune responses with viral evolution should address this central question, because it may well hold the key for successful immunotherapeutic interventions. Nevertheless, the therapeutic use of antibodies against PD-1 and CTLA-4, which is currently being tested in malignant disease, is also highly promising in chronic hepatitis C, particularly in combination with antigen-specific immunotherapy.

References

Alatrakchi, N., Graham, C.S., van der Vliet, H.J., Sherman, K.E., Exley, M.A., Koziel, M.J., 2007. Hepatitis C virus (HCV)-specific CD8+ cells produce transforming growth factor beta that can suppress HCV-specific T-cell responses. J. Virol. 81, 5882–5892.

Barber, D.L., Wherry, E.J., Masopust, D., Zhu, B., Allison, J.P., Sharpe, A.H., Freeman, G.J., Ahmed, R., 2006. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439, 682–687.

Boettler, T., Spangenberg, H.C., Neumann-Haefelin, C., Panther, E., Urbani, S., Ferrari, C., Blum, H.E., von Weizsacker, F., Thimme, R., 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., Demin, F., Capozzi, M., Carducci, F.C., Drapeau, C.M., Rocchi, G., Bergamini, A., 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.

Bowen, D.G., Shoukry, N.H., Grakoui, A., Fuller, M.J., Cawthon, A.G., Dong, C., Hasselschwert, D.L., Brasky, K.M., Freeman, G.J., Seth, N.P., Wucherpfennig, K.W., Houghton, M., Walker, C.M., 2008. Variable patterns of programmed death-1 expression on fully functional memory T cells after spontaneous resolution of hepatitis C virus infection. J. Virol. 82, 5109–5114.

Bowen, D.G., Walker, C.M., 2005. Mutational escape from CD8+ T cell immunity: HCV evolution, from chimpanzees to man. J. Exp. Med. 201, 1709–1714.

Cabrera, R., Tu, Z., Xu, Y., Firpi, R.J., Rosen, H.R., Liu, C., Nelson, D.R., 2004. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology 40, 1062–1071.

Chang, K.M., 2007. Regulatory T cells in hepatitis C virus infection. Hepatol. Res. 37 (Suppl 3), S327–330.

Chang, K.M., Gruener, N.H., Southwood, S., Sidney, J., Pape, G.R., Chisari, F.V., Sette, A., 1999. Identification of HLA-A3 and -B7-restricted CTL response to hepatitis C virus in patients with acute and chronic hepatitis C. J. Immunol. 162, 1156–1164.

Cox, A.L., Mosbruger, T., Lauer, G.M., Pardoll, D., Thomas, D.L., Ray, S.C., 2005a. Comprehensive analyses of CD8+ T cell responses during longitudinal study of acute human hepatitis C. Hepatology 42, 104–112.

Cox, A.L., Mosbruger, T., Mao, Q., Liu, Z., Wang, X.H., Yang, H.C., Sidney, J., Sette, A., Pardoll, D., Thomas, D.L., Ray, S.C., 2005b. Cellular immune selection with hepatitis C virus persistence in humans. J. Exp. Med. 201, 1741–1752.

Cucchiarini, M., Kammer, A.R., Grabscheid, B., Diepolder, H.M., Gerlach, T.J., Gruner, N., Santantonio, T., Reichen, J., Pape, G.R., Cerny, A., 2000. Vigorous peripheral blood cytotoxic T cell response during the acute phase of hepatitis C virus infection. Cell. Immunol. 203, 111–123.

Day, C.L., Seth, N.P., Lucas, M., Appel, H., Gauthier, L., Lauer, G.M., Robbins, G.K., Szczepiorkowski, Z.M., Casson, D.R., Chung, R.T., Bell, S., Harcourt, G., Walker, B.D., Klenerman, P., Wucherpfennig, K.W., 2003. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. J. Clin. Invest. 112, 831–842.

Day, C.L., Kaufmann, D.E., Kiepiela, P., Brown, J.A., Moodley, E.S., Reddy, S., Mackey, E.W., Miller, J.D., Leslie, A.J., DePierres, C., Mncube, Z., Duraiswamy, J., Zhu, B., Eichbaum, Q., Altfeld, M., Wherry, E.J., Coovadia, H.M., Goulder, P.J., Klenerman, P., Ahmed, R., Freeman, G.J., Walker, B.D., 2006. PD-1 expression on HIV-specific

T cells is associated with T-cell exhaustion and disease progression. Nature 443, 350–354.
Diepolder, H.M., Zachoval, R., Hoffmann, R.M., Wierenga, E.A., Santantonio, T., Jung, M.C., Eichenlaub, D., Pape, G.R., 1995. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. Lancet 346, 1006–1007.
Diepolder, H.M., Gerlach, J.T., Zachoval, R., Hoffmann, R.M., Jung, M.C., Wierenga, E.A., Scholz, S., Santantonio, T., Houghton, M., Southwood, S., Sette, A., Pape, G.R., 1997. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. J. Virol. 71, 6011–6019.
Dolganiuc, A., Paek, E., Kodys, K., Thomas, J., Szabo, G., 2008. Myeloid dendritic cells of patients with chronic HCV infection induce proliferation of regulatory T lymphocytes. Gastroenterology 135, 2119–2127.
Ebinuma, H., Nakamoto, N., Li, Y., Price, D.A., Gostick, E., Levine, B.L., Tobias, J., Kwok, W.W., Chang, K.M., 2008. Identification and in-vitro expansion of functional antigen-specific CD25+FoxP3+ regulatory T-cells in hepatitis C virus infection. J. Virol..
Franzese, O., Kennedy, P.T., Gehring, A.J., Gotto, J., Williams, R., Maini, M.K., Bertoletti, A., 2005. Modulation of the CD8+-T-cell response by CD4+ CD25+ regulatory T cells in patients with hepatitis B virus infection. J. Virol. 79, 3322–3328.
Gane, E.J., 2008. The natural history of recurrent hepatitis C and what influences this. Liver Transpl. 14 (Suppl. 2), S36–44.
Gerlach, J.T., Diepolder, H.M., Jung, M.C., Gruener, N.H., Schraut, W.W., Zachoval, R., Hoffmann, R., Schirren, C.A., Santantonio, T., Pape, G.R., 1999. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. Gastroenterology 117, 933–941.
Gerlach, J.T., Diepolder, H.M., Zachoval, R., Gruener, N.H., Jung, M.C., Ulsenheimer, A., Schraut, W.W., Schirren, C.A., Waechtler, M., Backmund, M., Pape, G.R., 2003. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 125, 80–88.
Golden-Mason, L., Palmer, B., Klarquist, J., Mengshol, J.A., Castelblanco, N., Rosen, H.R., 2007. Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. J. Virol. 81, 9249–9258.
Grakoui, A., Shoukry, N.H., Woollard, D.J., Han, J.H., Hanson, H.L., Ghrayeb, J., Murthy, K.K., Rice, C.M., Walker, C.M., 2003. HCV persistence and immune evasion in the absence of memory T cell help. Science 302, 659–662.
Gruener, N.H., Gerlach, T.J., Jung, M.C., Diepolder, H.M., Schirren, C.A., Schraut, W.W., Hoffmann, R., Zachoval, R., Santantonio, T., Cucchiarini, M., Cerny, A., Pape, G.R., 2000. Association of hepatitis C virus-specific CD8+ T cells with viral clearance in acute hepatitis C. J. Infect. Dis. 181, 1528–1536.
Hoofnagle, J.H., Seeff, L.B., 2006. Peginterferon and ribavirin for chronic hepatitis C. N. Engl. J. Med. 355, 2444–2451.
Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science 299, 1057–1061.
Kaplan, D.E., Ikeda, F., Li, Y., Nakamoto, N., Ganesan, S., Valiga, M.E., Nunes, F.A., Rajender Reddy, K., Chang, K.M., 2008. Peripheral virus-specific T-cell interleukin-10 responses develop early in acute hepatitis C infection and become dominant in chronic hepatitis. J. Hepatol. 48, 903–913.
Kasprowicz, V., Schulze Zur Wiesch, J., Kuntzen, T., Nolan, B.E., Longworth, S., Berical, A., Blum, J., McMahon, C., Reyor, L.L., Elias, N., Kwok, W.W., McGovern, B.G., Freeman, G., Chung, R.T., Klenerman, P., Lewis-Ximenez, L., Walker, B.D., Allen, T.M., Kim, A.Y., Lauer, G.M., 2008. High level of PD-1 expression on hepatitis C virus (HCV)-specific CD8+ and CD4+ T cells during acute HCV infection, irrespective of clinical outcome. J. Virol. 82, 3154–3160.
Kaufmann, D.E., Kavanagh, D.G., Pereyra, F., Zaunders, J.J., Mackey, E.W., Miura, T., Palmer, S., Brockman, M., Rathod, A., Piechocka-Trocha, A., Baker, B., Zhu, B., Le Gall, S., Waring, M.T., Ahern, R., Moss, K., Kelleher, A.D., Coffin, J.M., Freeman, G.J., Rosenberg, E.S., Walker, B.D., 2007. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nat. Immunol. 8, 1246–1254.
Kinter, A., McNally, J., Riggin, L., Jackson, R., Roby, G., Fauci, A.S., 2007a. Suppression of HIV-specific T cell activity by lymph node CD25+ regulatory T cells from HIV-infected individuals. Proc. Natl. Acad. Sci. U.S.A. 104, 3390–3395.
Kinter, A.L., Horak, R., Sion, M., Riggin, L., McNally, J., Lin, Y., Jackson, R., O'Shea, A., Roby, G., Kovacs, C., Connors, M., Migueles, S.A., Fauci, A.S., 2007b. CD25+ regulatory T cells isolated from HIV-infected individuals suppress the cytolytic and nonlytic antiviral activity of HIV-specific CD8+ T cells in vitro. AIDS Res. Hum. Retroviruses 23, 438–450.
Klade, C.S., Wedemeyer, H., Berg, T., Hinrichsen, H., Cholewinska, G., Zeuzem, S., Blum, H., Buschle, M., Jelovcan, S., Buerger, V., Tauber, E., Frisch, J., Manns, M.P., 2008. Therapeutic vaccination of chronic hepatitis C nonresponder patients with the peptide vaccine IC41. Gastroenterology 134, 1385–1395.
Lamonaca, V., Missale, G., Urbani, S., Pilli, M., Boni, C., Mori, C., Sette, A., Massari, M., Southwood, S., Bertoni, R., Valli, A., Fiaccadori, F., Ferrari, C., 1999. Conserved hepatitis C virus sequences are highly immunogenic for CD4(+) T cells: implications for vaccine development. Hepatology 30, 1088–1098.
Lang, K., Weiner, D.B., 2008. Immunotherapy for HCV infection: next steps. Expert Rev. Vaccines 7, 915–923.
Lauer, G.M., Barnes, E., Lucas, M., Timm, J., Ouchi, K., Kim, A.Y., Day, C.L., Robbins, G.K., Casson, D.R., Reiser, M., Dusheiko, G., Allen, T.M., Chung, R.T., Walker, B.D., Klenerman, P., 2004. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. Gastroenterology 127, 924–936.
Lauer, G.M., Lucas, M., Timm, J., Ouchi, K., Kim, A.Y., Day, C.L., Wiesch, J.S., Paranhos-Baccala, G., Sheridan, I., Casson, D.R., Reiser, M., Gandhi, R.T., Li, B., Allen, T.M., Chung, R.T., Klenerman, P., Walker, B.D., 2005. Full-breadth analysis of CD8+ T-cell responses in acute hepatitis C virus infection and early therapy. J. Virol. 79, 12979–12988.
Lauer, G.M., Ouchi, K., Chung, R.T., Nguyen, T.N., Day, C.L., Purkis, D.R., Reiser, M., Kim, A.Y., Lucas, M., Klenerman, P., Walker, B.D., 2002. Comprehensive analysis of CD8(+)-T-cell responses against hepatitis C virus reveals multiple unpredicted specificities. J. Virol. 76, 6104–6113.
Lauer, G.M., Walker, B.D., 2001. Hepatitis C virus infection. N. Engl. J. Med. 345, 41–52.
Lechner, F., Gruener, N.H., Urbani, S., Uggeri, J., Santantonio, T., Kammer, A.R., Cerny, A., Phillips, R., Ferrari, C., Pape, G.R., Klenerman, P., 2000a. CD8+ T lymphocyte responses are induced during acute hepatitis C virus infection but are not sustained. Eur. J. Immunol. 30, 2479–2487.
Lechner, F., Wong, D.K., Dunbar, P.R., Chapman, R., Chung, R.T., Dohrenwend, P., Robbins, G., Phillips, R., Klenerman, P., Walker, B.D., 2000b. Analysis of successful immune responses in persons infected with hepatitis C virus. J. Exp. Med. 191, 1499–1512.
Li, S., Jones, K.L., Woollard, D.J., Dromey, J., Paukovics, G., Plebanski, M., Gowans, E.J., 2007. Defining target antigens for CD25+ FOXP3 + IFN-gamma- regulatory T cells in chronic hepatitis C virus infection. Immunol. Cell. Biol. 85, 197–204.
Lucas, M., Ulsenheimer, A., Pfafferot, K., Heeg, M.H., Gaudieri, S., Gruner, N., Rauch, A., Gerlach, J.T., Jung, M.C., Zachoval, R., Pape, G.R., Schraut, W., Santantonio, T., Nitschko, H., Obermeier, M., Phillips, R., Scriba, T.J., Semmo, N., Day, C., Weber, J.N., Fidler, S., Thimme, R., Haberstroh, A., Baumert, T.F., Klenerman, P., Diepolder, H.M., 2007. Tracking virus-specific CD4+ T cells during and after acute hepatitis C virus infection. PLoS ONE 2, e649.
Maier, H., Isogawa, M., Freeman, G.J., Chisari, F.V., 2007. PD-1, PD-L1 interactions contribute to the functional suppression of virus-specific CD8+ T lymphocytes in the liver. J. Immunol. 178, 2714–2720.
Manigold, T., Racanelli, V., 2007. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. Lancet Infect. Dis. 7, 804–813.
Manigold, T., Shin, E.C., Mizukoshi, E., Mihalik, K., Murthy, K.K., Rice, C.M., Piccirillo, C.A., Rehermann, B., 2006. Foxp3+CD4+CD25+ T cells control virus-specific memory T cells in chimpanzees that recovered from hepatitis C. Blood 107, 4424–4432.
Missale, G., Bertoni, R., Lamonaca, V., Valli, A., Massari, M., Mori, C., Rumi, M.G., Houghton, M., Fiaccadori, F., Ferrari, C., 1996. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. J. Clin. Invest. 98, 706–714.
Nakamoto, N., Kaplan, D.E., Coleclough, J., Li, Y., Valiga, M.E., Kaminski, M., Shaked, A., Olthoff, K., Gostick, E., Price, D.A., Freeman, G.J., Wherry, E.J., Chang, K.M., 2008. Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. Gastroenterology 134, 1927–1937, 1937, e1921–1922.
Neumann-Haefelin, C., Frick, D.N., Wang, J.J., Pybus, O.G., Salloum, S., Narula, G.S., Eckart, A., Biezynski, A., Eiermann, T., Klenerman, P., Viazov, S., Roggendorf, M., Thimme, R., Reiser, M., Timm, J., 2008. Analysis of the evolutionary forces in an immunodominant CD8 epitope in hepatitis C virus at a population level. J. Virol. 82, 3438–3451.
Neumann-Haefelin, C., McKiernan, S., Ward, S., Viazov, S., Spangenberg, H.C., Killinger, T., Baumert, T.F., Nazarova, N., Sheridan, I., Pybus, O., von Weizsacker, F., Roggendorf, M., Kelleher, D., Klenerman, P., Blum, H.E., Thimme, R., 2006. Dominant influence of an HLA-B27 restricted CD8+ T cell response in mediating HCV clearance and evolution. Hepatology 43, 563–572.
Penna, A., Pilli, M., Zerbini, A., Orlandini, A., Mezzadri, S., Sacchelli, L., Missale, G., Ferrari, C., 2007. Dysfunction and functional restoration of HCV-specific CD8 responses in chronic hepatitis C virus infection. Hepatology 45, 588–601.
Radziewicz, H., Hanson, H.L., Ahmed, R., Grakoui, A., 2008a. Unraveling the role of PD-1/PD-L interactions in persistent hepatotropic infections: potential for therapeutic application? Gastroenterology 134, 2168–2171.
Radziewicz, H., Ibegbu, C.C., Fernandez, M.L., Workowski, K.A., Obideen, K., Wehbi, M., Hanson, H.L., Steinberg, J.P., Masopust, D., Wherry, E.J., Altman, J.D., Rouse, B.T., Freeman, G.J., Ahmed, R., Grakoui, A., 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.
Radziewicz, H., Ibegbu, C.C., Hon, H., Osborn, M.K., Obideen, K., Wehbi, M., Freeman, G.J., Lennox, J.L., Workowski, K.A., Hanson, H.L., Grakoui, A., 2008b. Impaired hepatitis C virus (HCV)-specific effector CD8+ T cells undergo massive apoptosis in the peripheral blood during acute HCV infection and in the liver during the chronic phase of infection. J. Virol. 82, 9808–9822.
Ray, S.C., Fanning, L., Wang, X.H., Netski, D.M., Kenny-Walsh, E., Thomas, D.L., 2005. Divergent and convergent evolution after a common-source outbreak of hepatitis C virus. J. Exp. Med. 201, 1753–1759.
Robertson, S.J., Hasenkrug, K.J., 2006. The role of virus-induced regulatory T cells in immunopathology. Springer Semin. Immunopathol. 28, 51–62.
Roncarolo, M.G., Gregori, S., 2008. Is FOXP3 a bona fide marker for human regulatory T cells? Eur. J. Immunol. 38, 925–927.
Rouse, B.T., Sarangi, P.P., Suvas, S., 2006. Regulatory T cells in virus infections. Immunol. Rev. 212, 272–286.
Rouse, B.T., Suvas, S., 2004. Regulatory cells and infectious agents: detentes cordiale and contraire. J. Immunol. 173, 2211–2215.
Sakaguchi, S., 2008. Regulatory T cells in the past and for the future. Eur. J. Immunol. 38, 901–937.
Sarrazin, C., Kieffer, T.L., Bartels, D., Hanzelka, B., Muh, U., Welker, M., Wincheringer, D., Zhou, Y., Chu, H.M., Lin, C., Weegink, C., Reesink, H., Zeuzem, S., Kwong, A.D., 2007. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 132, 1767–1777.

Shoukry, N.H., Cawthon, A.G., Walker, C.M., 2004. Cell-mediated immunity and the outcome of hepatitis C virus infection. Annu. Rev. Microbiol. 58, 391–424.
Shoukry, N.H., Grakoui, A., Houghton, M., Chien, D.Y., Ghrayeb, J., Reimann, K.A., Walker, C.M., 2003. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. J. Exp. Med. 197, 1645–1655.
Smyk-Pearson, S., Golden-Mason, L., Klarquist, J., Burton Jr., J.R., Tester, I.A., Wang, C.C., Culbertson, N., Vandenbark, A.A., Rosen, H.R., 2008. Functional suppression by FoxP3+CD4+CD25(high) regulatory T cells during acute hepatitis C virus infection. J. Infect. Dis. 197, 46–57.
Sprengers, D., Stoop, J.N., Binda, R.S., Kusters, J.G., Haagmans, B.L., Carotenuto, P., Artsen, A., van der Molen, R.G., Janssen, H.L., 2007. Induction of regulatory T-cells and interleukin-10-producing cells in non-responders to pegylated interferon-alpha therapy for chronic hepatitis B. Antivir. Ther. 12, 1087–1096.
Stauber, R.E., Kessler, H.H., 2008. Drugs in development for hepatitis C. Drugs 68, 1347–1359.
Stoop, J.N., van der Molen, R.G., Kuipers, E.J., Kusters, J.G., Janssen, H.L., 2007. Inhibition of viral replication reduces regulatory T cells and enhances the antiviral immune response in chronic hepatitis B. Virology 361, 141–148.
Suvas, S., Rouse, B.T., 2006. Treg control of antimicrobial T cell responses. Curr. Opin. Immunol. 18, 344–348.
Takahashi, T., Kuniyasu, Y., Toda, M., Sakaguchi, N., Itoh, M., Iwata, M., Shimizu, J., Sakaguchi, S., 1998. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int. Immunol. 10, 1969–1980.
Takaki, A., Wiese, M., Maertens, G., Depla, E., Seifert, U., Liebetrau, A., Miller, J.L., Manns, M.P., Rehermann, B., 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.
Thimme, R., Oldach, D., Chang, K.M., Steiger, C., Ray, S.C., Chisari, F.V., 2001. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J. Exp. Med. 194, 1395–1406.
Timm, J., Lauer, G.M., Kavanagh, D.G., Sheridan, I., Kim, A.Y., Lucas, M., Pillay, T., Ouchi, K., Reyor, L.L., Zur Wiesch, J.S., Gandhi, R.T., Chung, R.T., Bhardwaj, N., Klenerman, P., Walker, B.D., Allen, T.M., 2004. CD8 epitope escape and reversion in acute HCV infection. J. Exp. Med. 200, 1593–1604.
Ulsenheimer, A., Lucas, M., Seth, N.P., Tilman Gerlach, J., Gruener, N.H., Loughry, A., Pape, G.R., Wucherpfennig, K.W., Diepolder, H.M., Klenerman, P., 2006. Transient immunological control during acute hepatitis C virus infection: ex vivo analysis of helper T-cell responses. J. Viral. Hepat. 13, 708–714.
Urbani, S., Amadei, B., Tola, D., Massari, M., Schivazappa, S., Missale, G., Ferrari, C., 2006. PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. J. Virol. 80, 11398–11403.
Urbani, S., Amadei, B., Tola, D., Pedrazzi, G., Sacchelli, L., Cavallo, M.C., Orlandini, A., Missale, G., Ferrari, C., 2008. Restoration of HCV-specific T cell functions by PD-1/PD-L1 blockade in HCV infection: effect of viremia levels and antiviral treatment. J. Hepatol. 48, 548–558.
Vignali, D., 2008. How many mechanisms do regulatory T cells need? Eur. J. Immunol. 38, 908–911.
Ward, S.M., Fox, B.C., Brown, P.J., Worthington, J., Fox, S.B., Chapman, R.W., Fleming, K.A., Banham, A.H., Klenerman, P., 2007. Quantification and localisation of FOXP3+ T lymphocytes and relation to hepatic inflammation during chronic HCV infection. J. Hepatol. 47, 316–324.
Wedemeyer, H., He, X.S., Nascimbeni, M., Davis, A.R., Greenberg, H.B., Hoofnagle, J.H., Liang, T.J., Alter, H., Rehermann, B., 2002. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J. Immunol. 169, 3447–3458.