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TOPIC HIGHLIGHT

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Regulatory T cells in viral hepatitis

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Abstract

The pathogenesis and outcome of viral infections are significantly influenced by the host immune response. The immune system is able to eliminate many viruses in the acute phase of infection. However, some viruses, like hepatitis C virus (HCV) and hepatitis B virus (HBV), can evade the host immune responses and establish a persistent infection. HCV and HBV persistence is caused by various mechanisms, like subversion of innate immune responses by viral factors, the emergence of T cell escape mutations, or T cell dysfunction and suppression. Recently, it has become evident that regulatory T cells may contribute to the pathogenesis and outcome of viral infections by suppressing antiviral immune responses. Indeed, the control of HCV and HBV specific immune responses mediated by regulatory T cells may be one mechanism that favors viral persistence, but it may also prevent the host from overwhelming T cell activity and liver damage. This review will focus on the role of regulatory T cells in viral hepatitis.

Key words: Regulatory T cells; Viral hepatitis; Immunoregulation

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INTRODUCTION

An infection with HCV or HBV activates the immune system to defend the host with a broad range of innate and adaptive immune responses. Macrophages, natural killer cells and neutrophiles are an important part of the innate immune response that produces inflammatory and antiviral cytokines. Activated dendritic cells induce the differentiation of naïve T cells into virus specific CD4+ and CD8+ T cells for adaptive immunity. CD4+ T cells Type 2 induce B cells to produce antiviral antibodies; CD4+ T cells Type 1 cells secret IFN-γ and activate the massive proliferation of cytotoxic CD8+ cells that destroy infected cells or secret proinflammatory cytokines[1-3].

These complex molecular and cellular mechanisms help to control and, in the best case, to eliminate the virus in the acute phase of infection. However, in the majority of HCV and a significant amount of HBV infections the immune system fails to eliminate the virus and viral persistence is established. Although essential for successful virus elimination, the virus specific T cell responses of the host may also cause tissue damage and autoimmune reactions in the liver, especially in the setting of chronic viral infection. Therefore, many regulatory mechanisms of the immune system control the virus specific immune responses in order to prevent massive tissue damage or autoimmune disease.

Over the last few years, it has become evident that regulatory T cells (T_{reg} cells) may play an important role in the suppression of virus specific immune responses[4-6]. Indeed, several studies suggest a role of diverse populations of T_{reg} cells in the natural course of HCV and HBV infections. In this review, we will summarize the current knowledge about the role of regulatory T cells in HCV and HBV infection.

REGULATORY T CELL SUBSETS

A regulatory phenotype of a T cell population was first described for a CD4+ T cell subset that constitutively expresses the interleukin 2 receptor α-chain (CD25). In 1995 Sakaguchi *et al* showed that the transfer of lymphocytes depleted of CD4+CD25+ T cells into athymic mice caused the development of various autoimmune diseases in the recipient mice. Interestingly, the reconstitution with CD4+CD25+ T cells prevented autoimmune reactions in these mice, indicating a function of this T cell subpopulation in the control of self tolerance[7]. In the last decade numerous studies in mice and men showed that diverse T cell populations exhibit regulatory capacity and play an important role in the suppression of immune responses to self as well as foreign antigens[8]. Regulatory T cells are divided into a natural CD4+CD25+ T_{reg} cell population and diverse populations of induced or adaptive T_{reg} cells[9]. Natural T_{reg} cells develop in the thymus under strong TCR engagement with self peptides and play an important role in the maintenance of self-tolerance and immune homeostasis.

About 5%-10% of CD4+ T cells in mice and humans are natural T_{reg} cells[10]. Natural CD4+CD25+ T_{reg} cells constitutively express cytotoxic T-lymphocyte antigen 4 (CTLA4), glucocorticoid-induced TNF receptor family related protein (GITR)[11,12] and the forkhead family transcription factor FoxP3[13,14]. Of note, mutations in the gene *foxP3* cause the absence of natural T_{reg} cells and a loss of self tolerance. Indeed, it has been shown that FoxP3 expression is the essential factor for the induction of the natural T_{reg} cell population. FoxP3 is the best marker for the identification of natural T_{reg} cells in mice and men, thus far[15]. However, it has recently been shown that FoxP3 may also be transiently induced in activated human T cells[16,17].

In contrast to natural T_{regs} cells, induced T_{regs} cells develop from non regulatory T cells in the periphery and not in the thymus. Diverse populations of induced regulatory T cells have been identified, thus far. Among those, CD4+ cells secreting IL-10 or TGF-β, named T_R1- or T_H3 cells, as well as CD4+CD25+FoxP3+ T cells and CD8+ T cells with various phenotypes have been shown to have a regulatory capacity[8,18]. The extrathymically conversion of non-regulatory T cells into regulatory T cells requires special immunological conditions. In many respects, the cytokine TGF-β and a distinct mode of antigen exposure have been shown to play an important role in the development of induced T_{reg} cells[8,19]. Indeed, several studies in both mice and humans demonstrated that naïve and memory CD4+ T cells can be converted into CD4+CD25+FoxP3+ regulatory T cells in the presence of TGF-β[20-23]. In addition, it has been shown in several mouse models that a specific way of antigen presentation, e.g. continuous exposure to low dose antigen or to a systemic peripheral antigen, can lead to the expansion of induced CD4+CD25+FoxP3+ regulatory T cells[24-26].

Overall, these results and the phenotypical diversity of induced T_{reg} cell subpopulations indicate that several different mechanisms of T_{reg} cell development may exist in the periphery that still need to be defined for each subpopulation of induced T_{reg} cells. Furthermore, lineage relationship and functional overlap of induced T_{reg} cells and natural T_{reg} cells still need to be characterized in detail.

FUNCTIONAL CHARACTERISTICS OF REGULATORY T CELLS

The major function of natural and induced T_{reg} cells is the suppression of immune responses to self or foreign antigens. Indeed, numerous studies in mice and humans showed that regulatory T cells suppress the proliferation, cytokine-production (IFN-γ, IL-2) and cytolytic activity of naïve and antigen specific CD4+ and CD8+ cells. In addition, T_{reg} cells are able to suppress the functions of antigen presenting cells and B cells[27]. T_{reg} cells may mediate their suppressive activity either through the secretion of anti-inflammatory cytokines like IL-10 or TGF-β, direct killing of the target cells or distinct cell-cell contact dependent mechanisms[27]. The surface molecules CTLA4 and GITR have been suggested to play a role in direct cell-cell contact mediated suppression. CTLA-4 expressed on T_{reg} cells may bind to CD80/CD86 expressed on antigen presenting cells to activate the IDO (indoleamine 2,3-dioxygenase) dependent generation of tryptophan. A decrease of free tryptophan reduces T cell activation[28,29]. The transfer of cAMP from T_{reg} to effector cells via gap junction contact has recently been proposed to be another possible mode of cell-cell contact mediated suppression. cAMP inhibits the proliferation and IL-2 secretion of T cells[30]. In addition, T_{reg} cells have also been shown to generate extracellular adenosine that suppresses T cells responses[31]. However, the mechanisms and the antigen-specificity of T_{reg} cell mediated immunosuppression are still largely unknown.

In addition to the ability to control immune responses, at least those T_{reg} cell populations that express FoxP3 share other functional characteristics that distinguish them from effector T cells. Indeed, the expression of the transcription factor FoxP3 significantly influences the phenotype and function of T_{reg} cells[32]. FoxP3+ T cells show different TCR signaling patterns from effector T cells, do not proliferate well, when cultured *in vitro* and do not produce IL-2 or other inflammatory cytokines[32]. Recently, genome wide analysis of FoxP3 target genes in mouse natural T_{reg} cells revealed that FoxP3 regulates those genes involved into TCR signaling pathways and cytokine-production as well as genes encoding for T_{reg} cell associated surface molecules like CD25 or GITR[33,34]. In conclusion, the functional as well as the phenotypical differences between T_{reg} cells and effector T cells are largely dependent on the expression of FoxP3 in regulatory T cells.

REGULATORY T CELLS IN VIRAL INFECTION

Many viruses, like HCV and HBV, are able to evade the host immune response and to establish chronic infection. Efficient virus specific T cell responses are critical to eliminate the virus in the acute phase of infection. Importantly however, viruses have evolved strategies of immune evasion to subvert innate and adaptive immune responses and to facilitate viral persistence[35].

Growing evidence suggests that regulatory T cells may play an important role in the suppression of antiviral T cell responses in the acute and chronic phase of infection. Indeed, the virus specific induction of regulatory T cells may have two very different consequences: first, it may help the virus to establish viral persistence and second, it may be an important process that occurs to prevent excessive immunopathological damage[4,5].

First evidence that regulatory T cells may play a role in viral infection was forthcoming from a study by Souvas *et al* who showed that the depletion of CD4+CD25+ T cells in mice infected with herpes simplex virus (HSV) enhanced virus specific CD8+ T cell activity in the acute phase of infection as well as after viral clearance. Furthermore, HSV infection appeared to have a direct effect on natural T_{reg} cells, since regulatory T cells from HSV infected mice showed an increased suppressive capacity towards HSV specific and unspecific CD8+ T cell responses[36]. While these findings indicate a detrimental role of natural T_{reg} in the host-virus immune balance, another study with HSV infected mice proposed a

protective role of natural T_{reg} cells in viral infection. Mice suffering from blinding keratitis, caused by HSV infection, showed much more severe lesions in the eyes when depleted of natural CD4+CD25+ regulatory T cells[37].

Regulatory T cells have also been shown to play a role in chronic retroviral infections, indicated by studies with mice infected with Friend virus (FV). CD8+ T cells are critical for clearance of FV in the acute phase of infection, while insufficient CD8+ T cell effector functions are associated with viral persistence. A study by Dittmer *et al* showed that immunosuppression and CD8+ T cell impairment in chronic FV infection is a result of induced T_{reg} cell activity. Indeed, adoptive transfer experiments revealed that IL-10 secreting CD4+ cells from persistently infected mice suppressed antigen specific IFN-γ secretion of CD8+ cells[38]. Furthermore, a kinetic analysis of T cell responses in acute FV infection demonstrated that the onset CD8+ T cell dysfunction as early as 2 wk after infection was associated with the expansion of induced T_{reg} cells[39].

Taken together, these *in vivo* results from different mouse models suggest the important role of regulatory T cells in the suppression of virus specific T cell responses. In the following, recent human studies indicating the role of regulatory T cells in viral hepatitis will be discussed in more detail.

REGULATORY T CELLS IN HCV INFECTION

About 170 million people worldwide suffer from chronic hepatitis C virus infection. HCV is able to persist in up to 70% of those infected and may cause chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. HCV is a positive-stranded RNA virus that belongs to the Flavi viruses[1].

HCV clearance is associated with a vigorous HCV specific CD4+ and CD8+ T cell response in the acute phase of infection. In contrast, viral persistence is associated with a weak and dysfunctional virus specific T cell response[40]. Several possible mechanisms of T cell failure and HCV immune evasion have been proposed, like T cell deletion, T cell dysfunction and the emergence of viral escape mutations[2,41]. Recently, the possible role of different regulatory T cell populations in HCV persistence has also been suggested.

Indeed, several groups have shown a higher frequency of CD4+CD25+ regulatory T cells in the blood of chronically HCV infected persons versus recovered or healthy persons[42-45] and the presence of CD4+FoxP3+ T cells in the liver of chronically HCV infected patients[45,46]. However, whether these cells are natural T_{reg} cells that originate from the thymus or whether these cells are induced T_{regs} that developed from conventional CD4+ T cells upon HCV antigen contact in the periphery, remains to be determined. Of note, CD4+CD25+ regulatory T cells from chronically HCV infected patients are capable of suppressing HCV specific CD8 T cell and CD4+ T cell proliferation as well as CD8+ T cell IFN-γ secretion in a dose-dependent manner that requires direct cell-cell contact. However, CD4+CD25+ T_{reg} cells of infected subjects did not only suppress HCV specific T cell proliferation, but also Influenza-, CMV-, and EBV specific T cell responses, suggesting an antigen unspecific inhibition of CD8+ T cells[42,45].

The role of immunosuppressive cytokines, such as IL-10 and TGF-β, in the CD4+CD25+ T_{reg} cells dependent suppression remains controversial. Some studies reported that T_{reg} cells secrete IL-10 and TGF-β after HCV antigen stimulation and that TGF-β neutralization reverses T_{reg} cell mediated suppression of virus specific T cell responses[43,47], while others did not observe this effect[42,45].

It is important to note that CD4+CD25+ T_{reg} cells obtained from chronically HCV infected patients had an increased suppressive activity against HCV specific CD8+ T cells compared to natural T_{reg} cells isolated from subjects that had recovered from acute HCV infection, suggesting that chronic HCV infection leads to the expansion and activation of CD4+CD25+ T_{reg} cells. However the suppressive effect observed in patients who successfully cleared the virus was still significant [42]. Furthermore, a recent study with HCV infected chimpanzees, the only animal model for HCV infection, showed that the frequency of CD4+CD25+FoxP3+ T_{reg} cells and the suppressive capacity of those cells against virus specific T cell responses were as high in HCV recovered chimpanzees as in persistently HCV infected chimpanzees[48]. These results suggest that CD4+CD25+ T_{reg} cells do not only suppress virus specific T cell responses in chronic infection but may also control memory T cells after virus recovery. Of note, further analysis of CD4+CD25+ T_{reg} cells in chimpanzees revealed that T_{reg} cells from chronically HCV infected and recovered chimpanzees displayed fewer T cell receptor excision cycles and a better proliferative capacity *in vitro* compared to T_{reg} cells from HCV naïve chimpanzees. These data may indicate HCV specific proliferation of T_{reg} cells in HCV infected chimpanzees.

First evidence that T_{reg} cells may be induced by HCV antigens was provided by a study that examined the CD4+ T cell response to the HCV core protein. Interestingly, HCV specific IL-10 secreting T cells were detected in the blood of chronic HCV infected persons[49]. These regulatory T_R1 cells recognized the same epitopes on the core protein as IFN-γ producing T_H1 cells. However, HCV specific IL-10 secretion is not limited to the CD4+ T cell subset, since IL-10 producing HCV specific CD8+ T cells were identified that suppressed IFN-γ secretion of CD8+ T cells targeting the same epitope as the IL-10 secreting cells[50]. Of note, both cell subsets were predominantly present in the liver, suggesting a compartmentalization of effector and regulatory T cells to the site of infection. In addition, another study also reported the accumulation of HCV specific IL-10 producing CD8+ T cells in the liver of chronically infected patients. *In situ* staining of liver biopsies revealed that IL-10+ HCV specific CD8+ T cells are located in liver areas with low hepatocellular apoptosis and low liver fibrosis, further supporting a potential role of these cells in the prevention of liver damage[51]. However, these studies did not determine the FoxP3 expression of these CD8+ T cells.

The conclusion, that regulatory CD8+ T cells may play an important role in chronic HCV infection is further underlined by the observation that HCV specific CD8+

Table 1 Regulatory T cell subsets in HCV and HBV infection

Regulatory T cell phenotype	Virus	Compartment	Suppression	Cytokines
CD4+CD25+ (FoxP3+)[42-46,48,54,56,58,61,62]	HCV HBV	Blood/Liver	Cell-cell contact dependent suppression of antigen specific and unspecific T cell proliferation and cytokine production	In part IL-10/ TGF-β
CD4+ (T_R1 cells)[49]	HCV	Blood		IL-10
CD8+ (FoxP3$^+$)[50-52]	HCV	Blood/Liver	Suppression of antigen specific T cell proliferation and INF-γ secretion	IL-10/TGF-β
CD8+FoxP3+ CTLA-4+GITR$^+$[53]	HCV	Blood	Cell-cell contact suppression of antigen unspecific T cell proliferation	IFN-γ

CD25$^-$FoxP3$^-$ T cells from the blood of chronically infected patients suppress HCV specific T cell responses *via* TGF-β secretion. Of note, the blockade of TGF-β markedly enhanced the HCV specific IFN-γ secretion by CD4+ and CD8+ T cells[52].

A population of HCV specific FoxP3+CD8+ T cells that suppressed CD4+ and CD8+ T cell proliferation in a cell-cell contact dependent manner has also been described[53]. Indeed, these cells expanded simultaneously with FoxP3-CD8+ effector T cells after *in vitro* HCV specific peptide stimulation of peripheral blood mononuclear cells (PBMC) from chronically HCV infected patients. These results suggest that stimulation with a defined viral antigen leads to the expansion of two distinct CD8+ T cell populations: FoxP3$^-$ effector as well as FoxP3+ regulatory T cells. FoxP3 expression on virus-specific CD8+ T cells *ex vivo* has not been shown, however. Of note, *in vitro* stimulation of PBMC from chronically HCV infected patients with HCV specific antigens also resulted in an expansion of HCV specific CD4+CD25+FoxP3+ regulatory T cells[54]. However, at least for the CD8+ T cell compartment this is not a HCV specific effect since the expansion of virus specific FoxP3+ regulatory CD8+ T cells after *in vitro* peptide stimulation was also detected in influenza specific CD8+ T cells.

In summary, all these studies suggest a role of different regulatory T cell populations in the pathogenesis of HCV infection (Table 1). The elevated frequency of CD4+CD25+ regulatory T cells in the blood of chronically HCV infected patients, the ability of these cells to suppress HCV specific T cell responses, the accumulation of FoxP3+ T cells in the liver as well as the existence of different HCV specific regulatory T cell populations, strongly indicate that HCV infection induces virus-specific regulatory T cells that may contribute to viral persistence by suppressing HCV specific T cell responses. The presence of T_{reg} cells, especially in the liver, may also protect the host from tissue damage. In this context, it is interesting to note, that one study showed that HCV infected patients with normal alanine transaminase (ALT) levels have an increased frequency of HCV specific TGF-β secreting CD4+CD25+ regulatory T cells combined with decreased liver inflammation compared to patients with elevated ALT levels[47].

REGULATORY T CELLS IN HBV INFECTION

Hepatitis B virus is a hepatotropic DNA virus infecting about 300 million people worldwide. About 5%-10% of acutely infected patients develop a persistent HBV infection that is associated with T cell hyporesponsiveness and dysfunctions[55]. Thus, T_{reg} cells may also play a role in HBV infection. Indeed, one study reported a high frequency of CD4+ CD25+ T_{reg} cells in the blood of chronically HBV infected subjects and an increase of HBV specific T cell proliferation after depletion of CD4+CD25+ T cells[56]. Although these data suggest a potential role of T_{reg} cells in mediating T cell dysfunction during chronic HBV infection, another study reported discrepant results[57]. In fact, neither a higher frequency nor an elevated suppressive capacity of CD4+CD25+ T_{reg} cells isolated from the blood of chronically infected patients compared to CD4+CD25+ T_{reg} cells from persons recovered from HBV infection, were observed in this study[57].

However, recent studies shed more light on these controversial results about the role of T_{reg} cells in HBV infection. Indeed, a broad analysis of the frequency and function of CD4+CD25+ T_{reg} cells in the blood and liver of patients with acute HBV, chronic HBV, chronic severe HBV and healthy controls revealed that only patients with a chronic severe HBV infection showed a significant higher level of CD4+CD25+ T_{reg} cells in the blood compared to patients with mild course of chronic HCV and acute HCV infection[58]. A significant accumulation of CD4+CD25+FoxP3+ T_{reg} cells in the liver was found in patients with chronic HBV and chronic severe HBV infection[58,59]. A positive correlation between HBeAg level, HBV DNA level and the frequency of CD4+CD25+ T_{reg} cells in the blood of chronically infected patients further supports the role of T_{reg} cells in HBV infection[58-60]. Finally, two studies showed the presence of HBcAg-specific CD4+CD25+FoxP3+ T_{reg} cells in chronically HBV infected patients[61,62]. Interestingly, HBcAg specific CD4+CD25+ T_{reg} cells declined in the blood of patients during acute exacerbation of hepatitis in the immunoactive phase in chronic HBV infection while the HBcAg specific CD8+ T cell frequency increased at the same time. These data indicate that HBV specific T_{reg} cells may suppress HBV specific CD8+ T cells responses during flares in chronic HBV infection and may thus contribute to protection from severe hepatitis[61].

The analysis of T_{reg} cells during acute HBV infection showed that T_{reg} cells may also play a role in this state of the infection. Indeed, the frequency of CD4+CD25+ T_{reg} cells was normal in the early acute phase of infection, increased during the convalescent phase and decreased

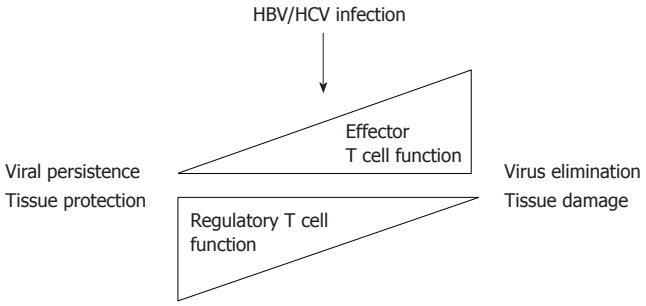

Figure 1 Regulatory T cells may contribute to viral persistence but they may also prevent tissue damage.

again to normal after virus resolution[58]. Of note, CD4+CD25+ T_{reg} cells isolated from the blood of acutely HBV infected patients had an increased suppressive activity against HBV specific T cell responses compared to antigen unspecific T cell responses, suggesting the generation of HBV antigen specific T_{reg} cells during acute HBV infection[58]. In summary, as in HCV infection, recent studies suggest that regulatory T cells do also play a role in HBV infection (Table 1).

FUTURE PERSPECTIVES

Taken together, there is strong evidence that different regulatory T cell populations mediate virus-specific T cell suppression in HCV and HBV infection. This immune suppression may contribute to viral persistence, but also to protection from overwhelming liver damage (Figure 1). Although, the studies discussed in this review give important first insights into the role of regulatory T cells in viral hepatitis, they also raise many questions. In fact, HCV infection in particular seems to induce a striking number of distinct HCV specific regulatory T cell populations. However, the lineage relationship between these T_{reg} cell populations as well as their relationship with HCV specific effector T cells remains elusive. It is unclear whether the CD4+CD25+ T_{reg} cells analyzed in HCV infected patients natural T_{reg} cells derive from the thymus develop from CD4+ T cells upon HCV antigen encounter in the periphery. The latter is most likely for HCV specific regulatory CD8+ T cells. However, if HCV specific T_{reg} cells are induced in the periphery, do they develop from naïve T cells as distinct induced CD4+ or CD8+ regulatory T cell lineages or do they generate from HCV specific effector memory T cells in a certain immunological context of the virus infection?

Other important questions that need to be addressed are the place and time point of regulatory T cell induction in HCV and HBV infection, as well as the mode and antigen specificity of T_{reg} cell mediated suppression in both viral infections. In addition, the presence and action of regulatory T cells in the liver, the site of virus replication and chronic inflammation should be analyzed in more detail. Furthermore, it will be important to determine if virus specific regulatory T cells do only play a role in the progression of a chronic infection or if they are already activated during the acute phase of infection and contribute to the development of viral persistence, as has already been indicated in the case of HBV infection.

Of note, T_{reg} cells may serve as a potential target for therapeutic interventions. The depletion of regulatory T cells during an acute viral infection may have the therapeutic potential to prevent viral persistence. Furthermore, a manipulation of regulatory T cells may also improve vaccine efficiency. Studies with mouse models already suggest that depletion of CD4+ CD25+ T cells can help to resolve an infection[36] or to enhance the effect of a virus DNA vaccination[63,64]. However, a depletion of regulatory T cells in the setting of a chronic viral infection could also lead to massive tissue damage, if virus specific CD8+ T cells are no longer suppressed. Therefore, an application of these approaches in humans requires a more detailed knowledge about the exact interplay of regulatory T cells, viruses and virus specific immune responses. Otherwise, the overall function of regulatory T cells in the maintenance of immune homeostasis and self tolerance could be dangerously disturbed.

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