The dual role of regulatory T cells in hepatitis B virus infection and related hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) represents a significant global cause of cancer-related mortality. Hepatitis B virus (HBV)infection is a major etiologic factor leading to HCC. While noteworthy progress has been made in managing HBV replication, achieving a cure for HBV-related HCC (HBV-HCC) remains challenging, and the overall survival outcome for HCC remains suboptimal. HBV persistence is attributed to a myriad of mechanisms, encompassing both innate and adaptive immune responses. Regulatory T cells(Tregs) are pivotal in upholding immune tolerance and modulating excessive immune activation. During HBV infection, Tregs mediate specific T cell suppression, thereby contributing to both persistent infection and the mitigation of liver inflammatory responses. Studies have demonstrated an augmented expression of circulating and intrahepatic Tregs in HBV-HCC, which correlates with impaired CD8 $^{+}$ T cells function. Consequently, Tregs play a dual role in the context of HBV infection and the progression of HBV-HCC. In this comprehensive review, we discuss pertinent studies concerning Tregs in HBV infection, HBV-related cirrhosis and HCC. Furthermore, we provide valuable treatment strategies pertinent to liver cancer management.

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Abstract

Hepatocellular carcinoma (HCC) represents a significant global cause of cancer-related mortality. Hepatitis B virus (HBV)infection is a major etiologic factor leading to HCC. While noteworthy progress has been made in managing HBV replication, achieving a cure for HBV-related HCC (HBV-HCC) remains challenging, and the overall survival outcome for HCC remains suboptimal. HBV persistence is attributed to a myriad of mechanisms, encompassing both innate and adaptive immune responses. Regulatory T cells(Tregs) are pivotal in upholding immune tolerance and modulating excessive immune activation. During HBV infection, Tregs mediate specific T cell suppression, thereby contributing to both persistent infection and the mitigation of liver inflammatory responses. Studies have demonstrated an augmented expression of circulating and intrahepatic Tregs in HBV-HCC, which correlates with impaired $CD8^+$ T cells function. Consequently, Tregs play a dual role in the context of HBV infection and the progression of HBV-HCC. In this comprehensive review, we discuss pertinent studies concerning Tregs in HBV infection, HBV-related cirrhosis and HCC. Furthermore, we provide valuable treatment strategies pertinent to liver cancer management.

Keywords

hepatitis B virus, hepatocellular carcinoma, regulatory T cells, immunotherapy, immunosuppression mechanisms

Introduction

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family and specifically infects hepatocytes[1], leading to the development of acute or chronic hepatitis B infections. A staggering 250 million individuals worldwide are chronic carriers of this virus[2], experiencing either asymptomatic persistence or progression to severe outcomes, such as liver cirrhosis, liver failure and hepatocellular carcinoma (HCC)[3]. Alarming global statistics report 799 000 annual deaths attributed to HBV infection, with a significant proportion of cirrhosis and liver cancer cases being HBV-associated [4] . Particularly in China, a region with high hepatitis B prevalence, over 80% of HCC cases are linked to HBV infection[5].

HBV infection constitutes a highly intricate disease that involves intricate immune mechanisms, encompassing both innate and adaptive immune responses, with particular emphasis on the latter, which plays a pivotal role in controlling acute HBV infection. In contrast, chronic HBV infection is characterized by weakened T-cell response to HBV antigens[6]. It is evident that host immune response assumes a critical function in the pathogenesis of liver inflammation, liver fibrosis, and HCC[7]. Collectively, HBV infection is accompanied by a compromised immune system function, leading to hightened viral replication and progression of liver disease, ultimately culminating in the development of HCC as a long-term complication[8].

Tregs represent a distinct subset of $CD4^+$ T lymphocytes that serve as key guardians of immune tolerance and are essential in controlling excessive immune activation[9-11]. Tregs can be classified into two subsets based on their origin: natural regulatory T cells (nTregs) and induced regulatory T cells (iTregs)[12-15]. While nTregs arise in the thymus iTregs are derived from naive $CD4^+$ T lymphocytes under the influence of tolerogenic conditions and various factors, such as IL-10 and TGF-β [16]. Initially, the surface marker CD25 (IL-2 receptor α chain) was identified as a hallmark of Tregs[17]. Later, the transcription factor Foxp3 was recognized as a specific marker of Tregs in both rodents and humans[18-20]. These $CD4^+CD25^+Foxp3^+$Tregs constitute approximately 5-10% of the total $CD4^+$ helper T cells[21].The expression of Foxp3 confers functional and phenotypic distinctions between Tregs and non-Tregs[22]. Notably, ectopic expression of Foxp3 in conventional T cells (Tconvs) bestows them with Treg-like suppressive function in vivo and in vitro, indicating that the FOXP3 gene is a master regulator of Treg inhibitiory function [18, 20]. In human studies, Tregs have been identified through the use of cell surface markers CD127 and CD45RA (the naive T cell marker), in addition to CD25 and Foxp3[23-25]. Upon stimulation, naive Tregs ($CD4^+CD45RA^+Foxp3^{low}CD127^{low}$ cells) can differentiate into effector Tregs ($CD4^+CD45R^-Foxp3^{high}$cells), which exhibit hightened suppressive capabilities in vitro[11]. Tregs' impairment has been implicated in various autoimmune diseases, including

type 1 diabetes[26], arthritis[27],thyroiditis[28]. However, in HCC patients, both circulating and tumor infiltrating FoxP3$^+$ Tregs were found to be enriched[29], thus exerting suppressive effects on antitumor immunity. Given the dual role of Tregs in autoimmune diseases and HBV-related HCC (HBV-HCC), this review aims to comprehensively explore the associations between Tregs and HBV-related liver diseases. Acronyms and abbreviations used throughout this work are defined in Table 1.

Nature history of HBV infection

Patients infected with HBV can develop acute hepatitis, ranging from subclinical to icteric hepatitis, and even acute liver failure, which develops in approximately 1% of patients with acute hepatitis B(AHB) and jaundice[30]. In most cases, acute HBV infection is self-limited, and the risk of chronicity in immunocompetent adults is not more than 5%. In recovery, HBV surface antigen (HBsAg) is cleared followed by undetectable HBV DNA from serum[31]. However, the risk of chronic hepatitis B(CHB) is remarkably increased in newborn (up to 90%), whose immune system is thought to be immature[32]. The clinical course of CHB can be classified into four stages, reflecting the dynamic relationship between the host immune system and viral replication. The immune-tolerant (IT) or HBeAg-positive chronic infection phase is the first phase of CHB, characterized by high HBV DNA, positive HBeAg, normal alanine aminotransferase (ALT), and minimal or no liver damage. The phase is more common and prolonged in perinatally infected subjects. Studies have revealed that events involving the initiation and promotion of HCC, such as HBV integration and clonal hepatocyte expansion, may start in this early phase of chronic infection[33]. The second phase is named "Immune active or clearance HBeAg positive phase". During this phase, liver fibrosis can develop rapidly, with some progressing to cirrhosis or even liver failure. The third phase is characterized by the absence of HBeAg and the presence of anti-HBeAg, low serum HBV DNA levels, and normal ALT. This phase was termed "Inactive HBeAg negative phase". The final phase is the "Reactivation phase". The low replication phase can persist lifelong, but some patients may subsequently develop HBV DNA replication either spontaneously or triggered by active immunosuppression, with or without e-antigen seroconversion, elevated HBV DNA load, and persistent ALT levels or relapses[6, 8].

Suppressive mechanisms of Tregs

Tregs play a key role in maintaining peripheral tolerance, limiting inflammatory response and preventing autoimmune diseases. However, they also restrict beneficial responses such as anti-tumour immunity[34]. In the past few years, there has been a lot of research about Tregs. The mechanisms by which Tregs are involved in the immune response can be divided into two main categories, contact-dependent and contact-independent. The former include the following: (a) Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) is expressed on Tregs and effector CD4$^+$ and CD8$^+$ T cells, which is a co-inhibitory molecules. It interacts with B7 of antigen-presenting cells (APC), especially dendritic cells(DCs), with a higher affinity than CD28[35]. Its interaction with these ligands induces their trans-endocytosis which can downregulate the expression of B7 by DCs[36], and lead to the production of Indoleamine 2,3 dioxygenase (IDO), which can affect the function and activation of effector T cells (Teffs)[37-39]. A previous study showed that Treg-specific CTLA-4 deficiency is able to produce autoimmune disease[40]. (b) Just like CTLA-4, programmed death-1 (PD-1) delivers a negative signal when interacting with its ligands (PD-L1 and PD-L2), blocking T cell activation and function. Even more interestingly, it has been shown that PD-L1 ligation can induce Foxp3 expression and pTregs generation, and loss of PD-1 expression on Tregs is conducive to Tregs phenotype unstability[41, 42]. (c) CD73 and CD39 have been identified as novel immune checkpoint targets. They are responsible for hydrolyzing extracellular ATP and ADP to AMP (by CD39) and converting AMP to adenosine (by CD73), which binds to the cell surface A2A receptor of effector cells and thereby suppresses a T cell response[43-45]. There has been a study indicating that Foxp3 can upregulate CD73/CD39 in Tregs and thses two molecule are highly expressed on about 80% of Foxp3$^+$Tregs [46, 47]. (d) Lymphocyte activation gene-3 (LAG-3) expressed on the surface of Tregs, with a high homology with CD4, binds to MHC II molecules on DCs with a higher affinity. This interaction suppresses DCs maturation and function. In addition, LAG-3 may also interfere with TCR signaling[48]. T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) is highly expressed on Tregs, and it competes with CD226 for binding to common ligands CD112 and CD155

with higher affinity[49-51]. The interaction suppresses both the function of DCs and the activation of T cells. Further research demonstrated that the interaction of TIGIT with CD155 on human DCs inhibits IL-12p40 production and potentiates IL-10 secretion by regulating the phosphorylation of p38 and Erk[52]. T-cell immunoglobulin-3 (TIM-3) alongside TIGIT, LAG-3 represents the next generation of immune checkpoints in cancer immunotherapy. Studies demonstrate that Tim-3 has four different ligands : Galectin-9 (Gal-9), high-mobility group protein B1 (HMGB1), phosphatidylserine (PtdSer), and carcinoembryonic antigen cell adhesion molecule 1 (CEACAM-1)[53]. Tim-3^+ Tregs are related to more immuno-suppressive activity compared to Tim-3^-Tregs[54]. In addition to those molecules, Tregs can secret extracellular vesicles(EVs). EVs are considered mediators of intercellular communication, containing a range of contents, such as lipids, proteins, and nucleic acids and Tregs have been proven to release immunosuppressive EVs[55]. A few undiscovered genes might be important. However, there remain many challenges about whether a few mechanisms are important or whether different mechanisms are required in different diseases. On the other hand, Tregs can secret soluble mediators that contribute to their suppressive activity, such as, IL-10, TGF-β, and IL-35, has also been evidenced[56-59]. Tregs constitutively express CD25, the α-chain of the IL-2 receptor with a higher affinity for IL-2, which is crucial for the proliferation of T cells. Thus, Tregs can cause cell death[60]. In addition, Tregs can release cytotoxic granules (perforin and granzymes), leading to apoptosis of the target cell. Other mediators also play a role in the inhibitory function of Tregs, such as neuropilin-1, Amphiregulin, and interleukin-34 (IL-34)[61].(Figure.1)

Regulatory T cells in acute HBV infection

HBV does not directly cause liver cell injury. The outcomes after infection are closely related to the host immune response. Appropriate immune response can lead to viral clearance and recovery, excess immune response can lead to liver failure and inadequate immune response will result in sustained HBV infection[62]. During the acute phase of infection, the majority (>95%) of adult patients are able to clear HBV spontaneously. Understanding the mechanisms of successful host immune responses leading to viral clearance and the mechanism of immune failure in persistent infections, is of great importance. Innate immunity is the host's first line of defense against HBV infection. After infection, HBV employs immune evasion strategies to induce little or no innate immune responses[63]. Many studies demonstrated that adaptive immune response significantly affects HBV infection clearance during AHB, especially HBV-specific effector $CD8^+$ T cells[64]. An early CD4 T-cell response to HBV infection may be necessary to induce the CD8 T-cell response required to clear the infection[65]. During acute resolving hepatitis B, there is a robust, coordinated adaptive immune response with virus-specific $CD8^+$ T cells mediating clearance of infected cells, B cells secreting neutralizing antibodies against HBsAg that block further infection and $CD4^+$ T cells supporting effective viral clearance[66]. In chimpanzees, at least 90% of the viral DNA is eliminated from the liver during a typical HBV infection by noncytolytic processes such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)[67, 68]. However, $CD4^+CD25^+$ Tregs suppress the activation, proliferation, and IFN-γ production of $CD4^+$ and $CD8^+$ T cells in chronic HBV infection[69, 70].

Nirupma revealed that the frequencies of peripheral blood Tregs are higher in AHB patients than CHB patients and healthy control (HC)[71]. Peng reported that the frequency of $CD4^+$ $CD25^+$ Tregs in AHB patients was comparable to that in healthy controls, while it was significantly increased in CHB patients[72]. Xu revealed that in AHB patients, circulating $CD4^+CD25^+$Tregs frequency was initially low, and with time, the profile reversed to show an increased number of circulating Tregs during the convalescent phase and restored to normal levels after resolution[73].

Characteristics of the intrahepatic specific T-cell response against HBV in AHB patients have not been studied due to the risk of complications associated with liver biopsies. Therefore, research using animal models is required. Stross et al. found that the numbers of Tregs increase in the liver rapidly after the early stage of HBV replication in a mouse model. Tregs alleviated immune mediated liver damage by attenuating the antiviral activity of effector T cells, but did not affect the development of HBV-specific $CD8^+$T cells [74]. Kosinska et al. reported that male mice showed functionally suppressed woodchuck hepatitis virus-specific $CD8^+$ T-cell responses in the liver and notably higher numbers of intrahepatic Tregs than females.

Subsequently, they found that functional blockade of Tregs by anti-CD25 antibody during transient HBV infection contributed to increased numbers of $CD8^+$T cells. This was related to a significantly reduction in HBV/WHV viral loads. Highly effective depletion of Tregs in DEREG mice also improved HBV-specific T-cell responses and accelerated virus clearance, although at the expense of increased liver damage[75, 76]. A similar result was found in another study. The article reported that male mice were treated with a neutralizing anti-CD25 antibody to deplete Tregs. As expected, serum HBsAg levels were significantly decreased after Tregs depletion, showing that Tregs depletion enhanced HBsAg clearance[77]. The immunomodulatory role of Tregs during acute HBV infection is a double-edged sword. Tregs prevent severe inflammation and immunopathology but also interfere with viral clearance[78, 79].

As opposed to Tregs, Th17 cells can produce proinflammatory cytokine IL-17, and can participate in liver damage and viral clearance after HBV Infection[80]. Another study revealed that the dynamic changes in the frequencies of Th17 and Tregs and the Th17/Treg ratio may be associated with the outcome of AHB patients. Compared with the control group, the acute stage group showed significant increases in the frequencies of Th17 and Tregs. Compared with the acute stage group, the early recovery group showed a significant reduction in the frequency of Th17, a substantial increase in the frequency of Tregs, a significant decrease in the Th17/Treg ratio. Compared with the early recovery group, the full recovery group showed a slight increase in the frequency of Th17, a significant reduction in the frequency of Tregs, but which was significantly higher than that in the control group, and a slight increase in the Th17/Treg ratio, which showed no significant difference between this group and the control group. In the acute stage of AHB, HBsAg ,and HBeAg levels were positively correlated with Th17/Treg ratio [81].

Tregs in chronic hepatitis B virus infection

The role of Tregs in AHB seems different from CHB. Scientists have explored the number of Tregs in HBV-related liver disease, summarized in Table 2. Previous studies showed that circulating Tregs level was elevated and positively correlated with serum HBV load and HBsAg levels in CHB patients[56, 82, 83]. In most studies, elevated Tregs is relevant to persistent HBV infection, but the regulatory mechanism of Tregs levels remains unclear. In addition, Patients with hepatitis B envelope antigen (HBeAg)-positive CHB harbored a higher percentage of peripheral blood and intrahepatic Tregs than those with HBeAg-negative CHB[84, 85]. HBeAg has been considered to promote chronicity, this function might be partially accomplished by inducing Tregs. Compared with HC, Tregs were also accumulated in the liver tissues and peripheral blood of CHB patients and were associated with the severity of liver inflammation in CHB patients[73] [86, 87]. A recent study found that HLA-DQ^+ Tregs have more robust inhibitory functions than HLA-DQ^- Tregs. Reducing the HLA-DQ^+Tregs can enhance the antiviral immune response to clear the virus[88]. Others did not find difference in Treg frequencies between patients with chronic and resolved HBV infection[89]. Similarly, Feng et al. found that Tregs increased significantly in the HBV group, but no relationships existed between Tregs and HBV-DNA load[90]. Discrepant findings might be due to different cell surface markers of Tregs, different stages of HBV infection, and patient heterogeneity.

The exact mechanisms of Tregs upregulation in CHB is still obscure. Recently, in vitro experiments suggested that Furin and transforming growth factor-β1 (TGFβ1) formed a positive feedback loop to activate Tregs, Furin or TGFβ1 knockdown in Tregs promoted Teff cell proliferation, stimulated IL-2 and IFN-γ secretion, and inhibited HBV replication. Furthermore, furin or TGFβ1 depletion in Tregs can enhance the killing activity of cytotoxic T lymphocytes (CTLs) against HBV1.3P-HepG2 cells[58]. A study showed that the significant source of TGF-β in the liver is hepatic stellate cells (HSCs), which are activated during chronic inflammation[91]. Therefore, persistent infection with HBV might contribute to TGF-β production from HSCs and result in the differentiation of conventional $CD4^+$ T cells into iTregs[92]. Liu et al. reported that Inhibitors of DNA-binding 3 (Id3) are favorable for Tregs differentiation, which could further inhibit antiviral immunity to promote the chronicity of infection. Id is natural negative regulators of E proteins, which can initiate the transcription of many genes [93]. Autophagy, an intracellular process, plays a role in maintaining cell homeostasis and survival[94]. Enhanced autophagy was shown to favor Tregs expansion and function[95]. It has been shown that HMGB1 and its receptors was significantly upregulated in both peripheral blood and

intra-hepatic of CHB patients and HMGB1-induced autophagy could maintain cell survival and functional stability of CHB-Tregs[96].In addition, another study showed that Galectin-9 contributes to the expansion of the Tregs through galectin-9/Tim-3 interaction[97]. Additionally, plasmacytoid dendritic cells (pDC) from HBV-infected patients induced the generation of a higher proportion of $CD4^+CD25^+$ Tregs compared with healthy patients[98]. It was recently shown that 5-Aza-2'-deoxycytidine (5-aza-CdR), the general methylation inhibitor, can induce naive T-cells differentiate toward Tregs by mediating demethylation[99]. (Figure. 2) The mechanism by which Tregs are regulated still needs to be further explored.

4.1 Changes of Treg activity in acute on chronic hepatitis B

CHB can progress to acute-on-chronic liver failure (ACLF). It is characterized by acute deterioration of liver function, coagulopathy, and subsequent multiple organ failure with 28-d mortality in the Asia-Pacific and African regions [3, 100, 101]. A recent study revealed that HBV-ACLF patients had significantly decreased Tregs compared with HC and CHB. And they thought that the model for end-stage liver disease (MELD) score was negatively correlated with $CD4^+CD25^+$Tregs[102]. This characteristic may help to predict HBV-ACLF severity and indicate a prognosis response to guide the treatment of HBV-ACLF. Another study documented that no obvious distinction was founded of viral-specific Tregs between CHB and asymptomatic HBV carriers (ASC). But Treg/Th17 ratio was decreased in CHB patients compared with ASC[103]. As opposed to Tregs, Th17 cells can produce proinflammatory cytokine IL-17, and can participate in liver damage and viral clearance after HBV infection[80]. A few studies reported that circulating Tregs and Th17 were increased in CHB and related ACLF patients compared with HC. Furthermore, the highest frequency has been observed in active CHB patients, while Th17 cells were most abundant in ACLF patients. And they also found that the Treg/Th17 ratio gradually decreased with the progression of ACLF[90, 104, 105]. Liang et al. found that Tregs frequency increased gradually during the HBV-ACLF. However, Th17 frequency gradually increases during progression from CHB to ACLF, but decreases sharply from the peak point to the recovery point. In addition, they reported that an increased Treg/Th17 ratio was associated with the survival of ACHBLF patients[106]. Others have voiced similar views[107]. However, Zhang et al. found that there was a decrease in Tregs with a concomitant increase in Th17 cells in the peripheral blood of patients in the remission stage of ACLF when compared with patients in the progression stage, CHB patients, or normal controls[108]. An imbalance of Th17 to Tregs may be used as a prognostic marker to predict disease progression. Therefore, more research and evidence are needed in the future.

4.2 Tregs response to antiviral therapy

CHB infection is characterized by an impaired antiviral immune response. Tregs positively associated with the levels of serum HBV DNA and HBsAg in CHB patients. At present, antiviral drugs mainly include nucleoside/nucleotide analogs (NAs) and pegylated interferon (PEG-IFN) [109]. Previous studies revealed that IL-2, IFN-γ, TNF-α, and IL-4 increased in CHB patients after NA treatment, and the numbers of Tregs were reduced[110, 111]. However, in contrast to this opinion, Zhang et al. argue that Tregs ($CD4^+$ $CD25^+$) levels were lower in the group that responded to lamivudine treatment than in the group that did not respond. No significant change of Tregs was observed in the entecavir treatment group[112], which may be due to the difference in the specific markers selected for Tregs. Th17 cells mediate non-specific inflammation, and the Th17/Treg imbalance is associated with disease progression in patients with CHB infection[113]. Liu reported that the Th17/Treg($CD4^+$ $CD25^+CD127^{dim}$) ratio was reduced in the therapeutic response (TR) group which responded to NAs therapy, and no differences were founded between the TR group and HCs[114]. In addition, the frequency of $CD25^+CD4^+$ T cells reduced following Peg-IFN-α-2b therapy[115].

Tregs in the progression of HBV-induced liver fibrosis

The global prevalence of HBV infection in patients with liver cirrhosis (LC) was 42%[116]. Cirrhosis imposes a substantial health burden on many countries, which has increased at the global level since 1990, partly due to population growth and ageing[4].Many inflammatory factors were expressed during liver cirrhosis. Tregs suppresses the activation of T and other immune cells and thus inhibits inflammation[117]. Many studies demonstrate a very close relationship between increased Tregs and $CD8^+$T-cell impairment and poor

survival in HCC[118]. But the role of Tregs in developing liver fibrosis (LF) remains controversial.

Some studies reported that Tregs can activate HSC to promote LF. In turn, activated HSC upregulate the levels of Tregs via the PGE2/EP2 and EP4 pathway. And TGF-β produced by Tregs may also worsen fibrosis by activating HSC. However, Tregs are likely to be only a minor source of TGF-β in the liver[119, 120]. In addition, IL-10 produced by Tregs has an anti-fibrotic effect by inhibiting the activation and proliferation of HSC[121]. Instead, Deng et al. reported that Tregs had an anti-fibrosis function by improving the anti-cirrhosis activity of human amniotic mesenchymal stem cells (hAMSC) in a mouse model[117]. The different Tregs identification, species (human/mouse), and disease models might partially explain the different roles of Tregs in LF. As discussed above, Tregs and Th17 cells have a common origin and belong to $CD4^+$ T cell subsets. They have antagonistic effects on each other during inflammation. A study revealed that the frequencies of $CD4^+CD25^+$ Tregs and Th17 cells significantly increased in mouse liver fibrosis models. Elevated Tregs are induced to antagonize the Th17 cells. And Tregs directly downregulated the pro-fibrotic features of HSC[86]. Lan et al. found that circulating Tregs were reduced in LC compared with HC, Tregs and Th17 cells were negatively correlated[113]. Addtionally, many studies thought that Treg/Th17 ratio was negatively related to the severity of liver fibrosis[122-124]. Currently, it was limited research on the role of Tregs in HBV-LF. In the future, more studies are necessary to ascertain the exact function of Tregs, especially in the liver microenvironment.

Tregs in HBV-HCC

The interaction of Tregs with adaptive immune cells in HBV-HCC

HBV is the most common cause of HCC, with an estimated prevalence of 44%-55% of HCC cases worldwide[125]. Persistent HBV infection can lead to varying liver damage, eventually leading to hepatitis, fibrosis, cirrhosis, and HCC. The mechanism underlying HBV-HCC is not completely clear. As immunosuppressive cells, Tregs and their associated factors, such as metabolites and secreting cytokines, mediate the immune tolerance of the tumor microenvironment. A recent study reported that the HBV-HCC microenvironment is more immunosuppressive and exhausted than the non-viral-HCC microenvironment[126]. It is well-documented that HCC patients with a high lymphocyte infiltration level in the tumors have a lower risk of recurrence and a better prognosis[127]. The percentage of Tregs in the peripheral blood and intrahepatic is increased in HBV-HCC. Increased Tregs may impair $CD8^+$T cells and be associated with cancer progression and poor survival in HBV-HCC[128-130]. In addition, an elevated level of Tregs is accompanied by reduced infiltration of $CD8^+$T cells in tumor regions compared with nontumor regions. $CD8^+$T cells in HCC tumor tissues can differentiate into CTLs, which then produce cytokines (IFN-γ, TNF-α) and cytotoxic enzymes (perforin and granulosin B) to clear cancer cells[131]. However, the expression of granzyme A, granzyme B, and perforin was decreased significantly in tumor-infiltrating $CD8^+$T cells[128]. The exhausted $CD8^+$ effector T cells eventually weaken the tumor surveillance of the adaptive immune system and lead to the immune escape of tumor cells.

The proportion of Tregs in HBV-HCC microenvironment was significantly higher than that of non-virus-associated HCC, and the expression levels of inhibitory receptors PD-1 and LAG-3 were higher, showing a more potent immunosuppressive capability[126]. Qiu et al. demonstrated that Furin or TGFβ1 knockdown in Tregs promoted Teff cell proliferation and enhanced the killing activity of CTLs against HCC cells and HBV in vitro[58]. Nishida et al. reported that $CCR4^+$ Tregs were the principal type of Tregs in HBV-associated HCC, which were related to sorafenib resistance and HBV load titers. In addition, $CCR4^+$Tregs can express more IL-10 and IL-35 compared with $CCR4^-$ Tregs, which enhanced the ability of suppressing $CD8^+$ T cells[132]. $CCR4^+$ Tregs are recruited into tumor microenvironment by binding to CCL22 and CCL17 in HBV-HCC[133]. HBV-encoded gene HBx induces substantial IL8 production through activating MEK-ERK signal. The activity of IL8 is mainly determined by binding to its receptor CXCR1 and CXCR2. The IL8-CXCR1 axis on the hepatic vascular endothelium promotes the growth and dissemination of HCC by recruiting Tregs in the liver microenvironment[134]. Tregs play a vital role in HBV-related diseases. Thus, an in-depth investigation of factors that regulate Tregs functions is crucial for the better treatment of HBV-HCC and CHB.

The interaction of Tregs with innate immune cells in HBV-HCC

Natural killer (NK) cells are an essential component of innate immunity in the liver, the site of HBV replication[135]. The study revealed that $CD4^+CD25^+$ Tregs directly suppressed NK cell-mediated hepatocytotoxicity through membrane-bound TGF-β (mTGF-β) and OX40/OX40L interaction in HBV-associated liver disease[136]. In a mouse model, Liu et al. found that MicroRNA-15a/16-1 prevented Kupffer cells (KCs) from overproducing CCL22 by inhibiting nuclear factor-κB, which activates the transcription of CCL22. By reducing CCL22 binding to C-C chemokine receptor type 4 on Tregs, microRNA-15a/16-1 can impair Treg function[137]. Thus, microRNA-15a/16-1 prevents HCC by disrupting the communication between KCs and Tregs, which provides new ideas for the treatment of HCC. Under the hypoxic state.A large amount of triggering receptor expressed on myeloid cells-1 positive ($TREM\text{-}1^+$) tumor-associated macrophages(TAM) were enriched in tumor tissues. Wu et al. reported that $TREM\text{-}1^+$ TAMs abundant at advanced HCC indirectly impaired the killing function of $CD8^+$ T cells and induced $CD8^+$ T-cells apoptosis, and recruited $CCR6^+$ $Foxp3^+$ Tregs into tumor tissues by secreting a large number of chemokine CCL20 to promote the progression of HCC[138].

The myeloid-derived suppressor cells (MDSC) is a group of regulatory immune cell population residing in the liver. HBV can enhance the secretion of TGF-β and IL-10 by MDSC, and then induce the formation of Tregs. APCs that play a crucial role in activating T cell-mediated, antigen-specific adaptive immune responses. B7-H4 belongs to B7 family member, which can negatively regulate T cell responses[139]. Kryczek et.al showed that Tregs can induce APCs to produce high levels of IL-10 and, in turn, stimulate APC B7-H4 expression. Thus, APCs can suppress T cell activation via B7-H4 induction[140]. TIGIT was expressed on regulatory, memory and activated T cells. Poliovirus receptor (PVR, also called CD155), which is highly expressed on DCs, bound TIGIT with high affinity. TIGIT interacted with CD155 on DCs to enhance the secretion of IL-10 by DCs, which impedes $CD4^+$ T-cell proliferation and function[141]. In a mouse model of acute HBV infection, NK cells have been shown to be critical for HBV clearance[142]. NK cell dysfunction is associated with impaired $CD8^+$ T cell responses in CHB[143]. Ma et al. demonstrated that HBeAg induces IL-10 production in Tregs, which subsequently upregulates the expression of NKG2A on NK cells, leading to NK cell exhaustion, which in turn suppresses the anti-tumor immunity of organism and evades the monitoring of the immune system in HCC[144, 145]. (Figure.3)

Therapies targeting Tregs in HBV or HBV-HCC

Tregs play a critical role in maintaining peripheral tolerance, thus garnering substantial interest as a potential therapeutic approach for preventing autoimmunity and establishing transplantation tolerance[146]. However, in the context of HCC, immune suppression can facilitate immune escape of tumor cells. Over the past years, Tregs have been implicated in the suppression of virus-specific immune responses, thereby providing a mechanism for the persistence of HBV. Notebly, the depletion of Tregs during AHB has been shown to impede its transition to a chronic state[22]. Moreover, Tregs and associated factors play a key role in immunosuppressive tumor microenvironment of HCC, thus highlighting the potential of targeting Tregs to inhibit HCC progression. Immune checkpoint inhibitors (ICIs) are designed to target immune checkpoints that dampen immune cell activity and exhibit ant-tumor effects. Several immune checkpoints, such as TIM-3, TIGIT, or LAG-3 are expressed in Tregs, presenting promising avenues for future research in HCC treatment aimed at inhibiting Treg function [147, 148]. Non-virally associated HCC generally exhibits higher levels of IFN-γ, IL-17, Granzyme B, and TNFα, whereas virally-associated tumors have increased PD-1 expression on T cells, indicative of a more suppressive environment fostered by HBV infection [126].

Research indicated that Tregs induce immune suppression via the secretion of immunosuppressive cytokines such as IL-35, IL-10, and TGF-β. IL-35 can restrain the proliferation of HBV-specific CTL cells and IFN-γ secretion in vitro. IL-35 is associated with HBV-related hepatic complications, including CHB, cirrhosis, and HCC[59]. An elevated level of IL-10 is linked to HBV serum titers and the degree of liver inflammation[149]. A recent study showed that TGFβ1 knockdown in Tregs can inhibit HBV replication in vitro[58]. Therefore, blocking IL-10, IL-35, and TGFβ1 expression helps restore the impaired function of effector T cells. A recent study reported that $CCR4^+$Tregs were the predominant type of Tregs recruited to HBV-HCC, associating

with sorafenib resistance and HBV load titers. Targeting intratumoral $CCR4^+$ Tregs was shown to overcome sorafenib resistance and sensitize tumors to immune checkpoint blockade in mouse models of liver cancer[132]. Herein, severe autoimmunity could be avoided by selectively depleting intratumoral Tregs.

Recent studies demonstrated that blocking glycolysis can promote the generation of Tregs through the hypoxia-inducible factor 1α (HIF1α). The concentration of amino acids can affect the differentiation and function of Tregs. The metabolic pathways and factors influencing Tregs differentiation and function can be considered new therapeutic approaches during viral infection[150, 151]. It has been found that IL-15 were downregulated in CHB patients with type 2 diabetes mellitus. IL-15 can suppress Treg function and inhibit the expression of immune checkpoint molecules in Tregs[152]. Huang et al. showed that exosomal circGSE1 stemming from HCC cells promotes the progression of HCC by inducing Tregs expansion via regulating the miR-324-5p/TGFBR1/Smad3 axis[153]. In the future, exosomal circGSE1 may be used as a promising biomarker for immunotherapy of HCC. Other findings may open new avenues in developing therapeutic strategies to activate specific anti-HBV immunity by diminishing Tregs autophagy[96].

In conclusion, Tregs appear to play a pivotal role in the maintenance of HBV infection, HBV-related cirrhosis, and HCC. AS such, it becomes imperative to explore strategies targeting Tregs molecules and attenuating Treg activity during specific periods of HBV infection and HCC. Novel ICIs targeting molecules such as anti-TIM-3, anti-TIGIT, or anti-LAG-3 have demonstrated the ability to restore the functionality of tumor-infiltrating T cells in vitro[154] and are currently being evaluated in early-stage clinical trials.

Conclusions

Collectively, the role of Tregs in hepatitis viral infections appears to exhibit a dual nature. On one hand, Tregs mediate targeted suppression of T cells in the context of HBV infection, potentially contributing to viral persistence, while simultaneously safeguarding against excessive liver damage. However, it remains uncertain whether these divergent roles can be ascribed to the same Treg populations or if they involve distinct Treg subpopulations. Moreover, the underlying mechanisms governing these distinct mechanisms mediate these disparate functions requires further investigation. A promising avenue for enhancing HBV-related disease management involves the specific targeting of Tregs and a refined understanding of the optimal time frame for such intervention. In addition, Tregs have the ability to shape a tumor-prone immune microenvironment for HCC formation by weakening the immune surveillance function of the innate and adaptive immune systems. Consequently, strategies aimed at inhibiting Treg function and promoting an enhanced immune state within the tumor microenvironment hold considerable promise for the treatment of HCC. Exploring the modulation of Treg activity and its potential impact on HCC treatment merits further research and consideration.

CONFLICT OF INTEREST

The authors declare that they have no competing inter-

ests regarding the publication of this manuscript.

HCC	Hepatocellular carcinoma
HBV	Hepatitis B virus
HBV-HCC	HBV-related Hepatocellular carcinoma
Tregs	Regulatory T cells
nTregs	Natural regulatory T-cells
iTregs	Induced regulatory T cells
Tconvs	Conventional T cells
AHB	Acute hepatitis B
CHB	Chronic hepatitis B
IT	Immune-tolerant
ALT	Alanine aminotransferase
CTLA-4	Cytotoxic T-lymphocyte–associated antigen 4

HCC	Hepatocellular carcinoma
APC	Antigen-presenting cell
DCs	Dendritic cells
IDO	Indoleamine 2,3 dioxygenase
Teffs	Effector T cells
PD-1	Programmed death-1
LAG-3	Lymphocyte activation gene-3
TIGIT	T cell immunoreceptor with immunoglobulin and ITIM domain
TIM-3	T-cell immunoglobulin-3
Gal-9	Galectin-9
HMGB1	High-mobility group protein B1
PtdSer	Phosphatidylserine
CEACAM-1	Carcinoembryonic antigen cell adhesion molecule 1
EVs	Extracellular vesicles
HBsAg	HBV surface antigen
IFN-γ	Interferon-γ
TNF-α	Tumor necrosis factor-α
HC	Healthy control
HBeAg	Hepatitis B envelope antigen
IL	Interleukin
TGFβ1	Transforming growth factor-β1
CTLs	Cytotoxic T lymphocytes
HSCs	Hepatic stellate cells
Id3	DNA-binding 3
pDC	Plasmacytoid dendritic cells
5-aza-CdR	5-Aza-2'-deoxycytidine
ACLF	Acute-on-chronic liver failure
MELD	Model for end-stage liver disease
ASC	Asymptomatic HBV carrier
NAs	Nucleoside/nucleotide analogs
PEG-IFN	Pegylated interferon
TR	Therapeutic response
LF	Liver fibrosis
hAMSC	Human amniotic mesenchymal stem cells
LC	Liver cirrhosis
NK	Natural killer cell
mTGF-β	Membrane-bound TGF-β
KC	Kupffer cells
TREM-1	Triggering receptor expressed on myeloid cells-1
TAM	Tumor-associated macrophages
MDSC	Myeloid-derived suppressor cells
PVR	Poliovirus receptor
ICIs	Immune checkpoint inhibitors
HIF1α	Hypoxia-inducible factor 1α

Table 1 Detailed definitions of the main acronyms

Markers(Tregs)	Species	Position	Comparison (Frequency)	Study
$CD4^+CD25^+$	Human	PB	ACLF<HC,CHB	2021-[102]

Markers(Tregs)	Species	Position	Comparison (Frequency)	Study
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<ACLF<CHB	2021-[155]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<CHB	2020-[144] 2021-[156]
$CD4^+CD25^+FOXP3^+$	Human	PB,IH	HbeAg(-)CHB <HbeAg(+)CHB	2020-[84]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC>LC	2019-[113]
not described	Human	PB	HC<CHB,LC	2019-[124]
$CD4^+CD25^+CD127^{dim/-}$			ASC/CHB ns	2019-[103]
$CD4^+CD25^+FOXP3^+$	Human	PB	LC¡HC	2019-[103]
$CD4^+CD25^+FOXP3^+$	Human	IH	early LF<advanced LF	2017-[119]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<CHB,LC,HCC	2015- [90]
$FOXP3^+$	Human	IH	HBsAg(+)HCC> HBsAg(-)HCC	2015-[130]
$CD4^+CD25^+FOXP3^+$	Human	PB	ACLF<CHB	2014-[106]
$CD4^+CD25^{high}CD127^{low}$	Human	PB	HC<CHB CHB/HC ns CHB/LC ns	2014-[122]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<CHB<ACLF	2013-[107]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC,CHB,ACLF<AHB	2012-[105]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<CHB	2012-[86]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<HCC,CHB HCC/CHB ns HBsAg(+)HCC> HBsAg(-)HCC HBsAg(-)HCC/HC ns	2010-[129]
		IH	HC<CHB,HCC	
$CD4^+CD25^+FOXP3^+$	human	PB	CHB,HC<AHB	2009-[71]
$CD4^+CD25^+$	human	PB	HC,AHB<CHB	2008-[72]
$CD4^+CD25^+$	Human	PB	HC,AHB¡CHB	2008-[72]
$CD4^+CD25^+FOXP3^+$	Human	PB	HC<LC<HCC	2007-[128]
$CD4^+CD25^+$	Human	PB	HC=AHB <CHB	2006-[73]

Table2. Changes of Tregs in HBV-related liver disease

LC: liver cirrhosis; IH:intrahepatic; PB: peripheral blood; HbeAg: hepatitis B envelope antigen; ACLF: acute-on-chronic liver failure; LF: liver fibrosis; HCC: hepatocellular carcinoma; ASC :asymptomatic HBV carriers; ns: no significance

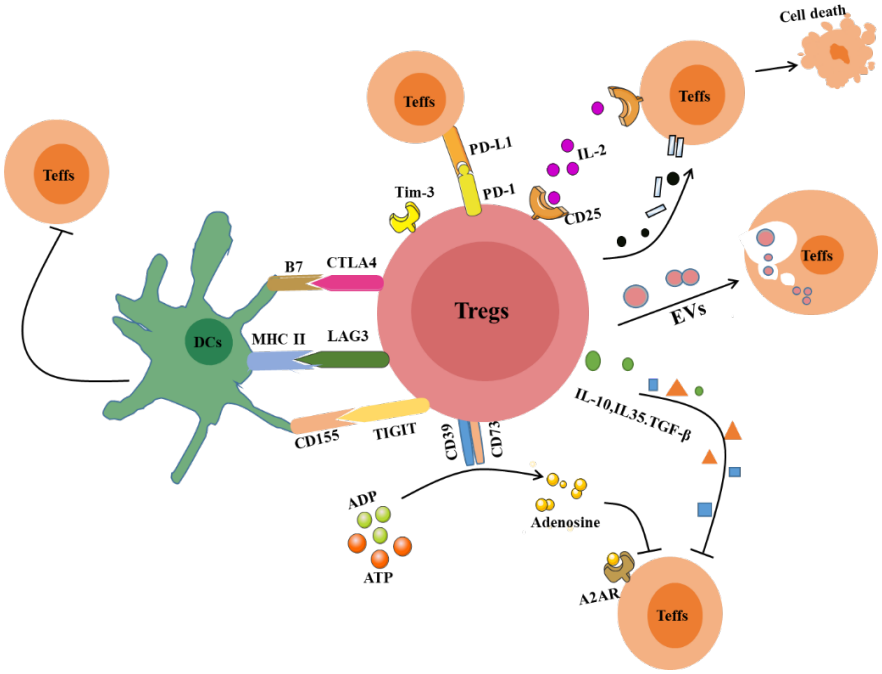

Figure 1. Mechanism of immune function of Tregs

Figure 2. Mechanism of Tregs elevation in CHB

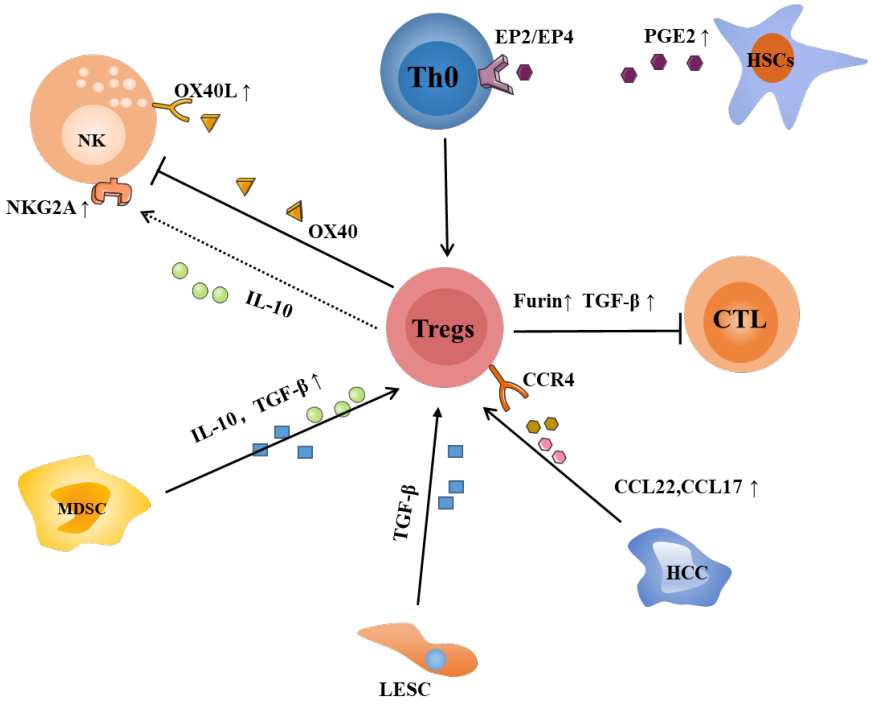

Figure 3. Interaction between Tregs and other cells in HBV-related liver disease

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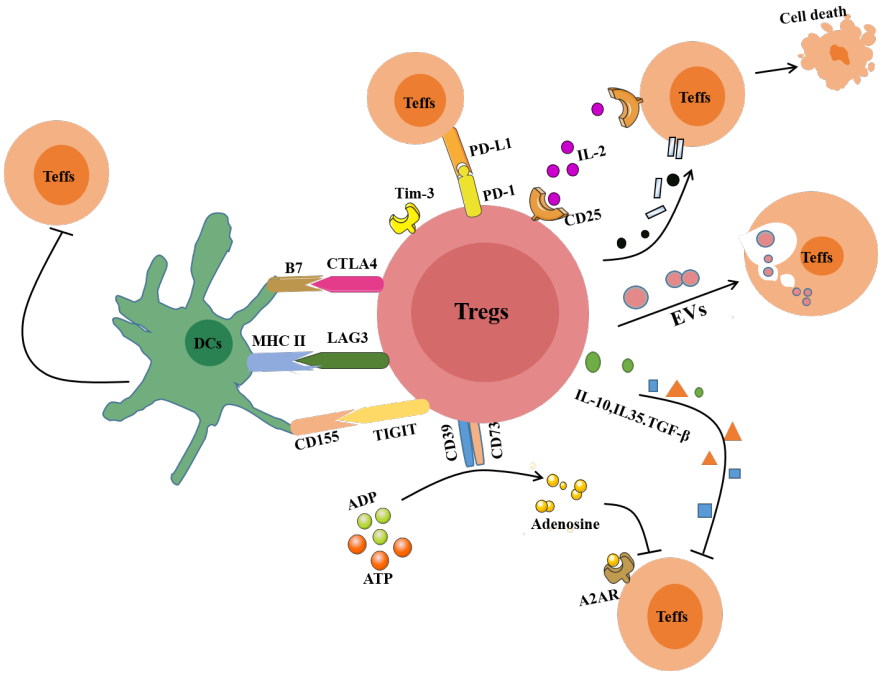

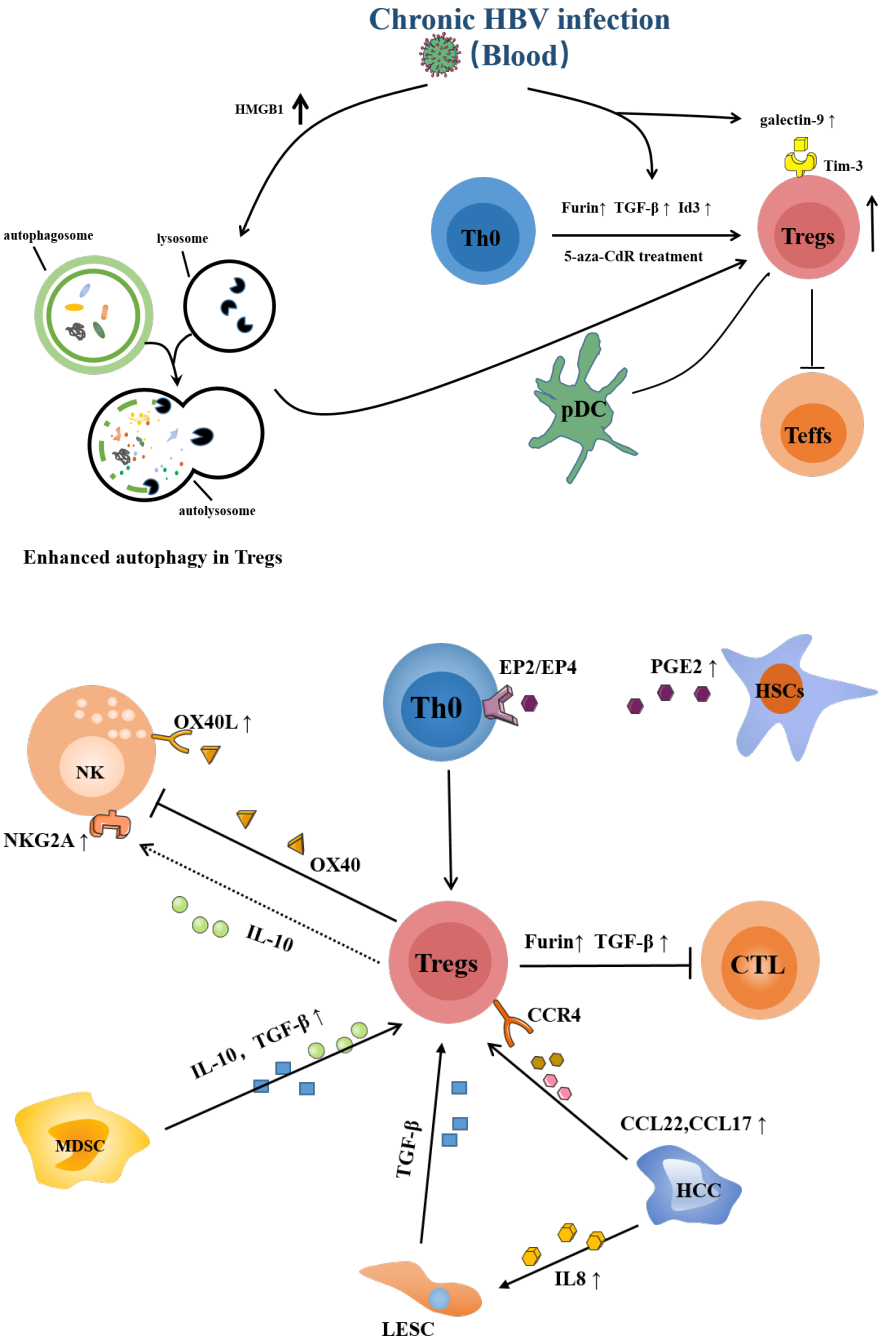

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