REVIEW

Adaptive immunity in the liver

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The anatomical architecture of the human liver and the diversity of its immune components endow the liver with its physiological function of immune competence. Adaptive immunity is a major arm of the immune system that is organized in a highly specialized and systematic manner, thus providing long-lasting protection with immunological memory. Adaptive immunity consists of humoral immunity and cellular immunity. Cellular immunity is known to have a crucial role in controlling infection, cancer and autoimmune disorders in the liver. In this article, we will focus on hepatic virus infections, hepatocellular carcinoma and autoimmune disorders as examples to illustrate the current understanding of the contribution of T cells to cellular immunity in these maladies. Cellular immune suppression is primarily responsible for chronic viral infections and cancer. However, an uncontrolled auto-reactive immune response accounts for autoimmunity. Consequently, these immune abnormalities are ascribed to the quantitative and functional changes in adaptive immune cells and their subsets, innate immunocytes, chemokines, cytokines and various surface receptors on immune cells. A greater understanding of the complex orchestration of the hepatic adaptive immune regulators during homeostasis and immune competence are much needed to identify relevant targets for clinical intervention to treat immunological disorders in the liver.

INTRODUCTION

The liver is one of the most vital organs in the human body and has specific physiological functions and a unique anatomical architecture. Blood circulates through the liver via the hepatic artery and portal vein. The hepatic artery shares arterial functions with other organs, whereas the hepatic portal vein contains a myriad of absorbed nutrients, microbial products and drugs from the gastrointestinal tract and spleen, some of which may be potential antigens. Before entering systemic circulation, the blood in the portal vein must be filtered through the liver, which is required to perform extensive metabolic functions and exert powerful immunocompetence, thus acting as a strong protective barrier between the portal vein and the systemic circulation. In fact, the liver is also known as 'lymphoid liver' because it harbors a large number of immune cells, such as leukocytes, dendritic cells (DCs), natural killer cells, T- and B cells. Moreover, in inflammation, some hepatic parenchymal cells may act as proinflammatory cells, as exemplified by hepatocytes and cholangiocytes.[1] From the perspective of the hepatic microenvironment, the architecture of the liver sinusoid is specifically designed to lack a basement membrane and to exhibit a very slow blood flow, which is conducive to the aggregation of various pathogens, oncocytes and immunocytes. These immunocytes are responsible for innate immunity and adaptive immunity to protect the human body from infections, tumors and even autoantigens in autoimmune liver diseases (AILDs) through highly orchestrated mechanisms.[2–5] In this review, we will focus on adaptive immunity and discuss[1] the major T-cell subgroups and their functions, and[2] the current understanding of the role of T cells in hepatic infections, liver tumors and AILDs, with a particular emphasis on common hepatic diseases, including viral infections (hepatitis B and C), hepatocellular carcinoma (HCC), primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH), to illustrate the complexity and challenges in these rapidly developing fields.

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MAJOR SUBSETS OF T CELLS AND THEIR FUNCTIONS IN ADAPTIVE IMMUNITY

On the basis of their phenotypes and functions, the major T lymphocytes that are involved in adaptive immunity include CD4 T cells, CD8 T cells and γδ-T cells, which are further categorized into several subsets (Table 1). CD4 T cells have at least five functional subsets, including helper T (Th)1, Th2, Th17 and follicular helper T (Tfh) cells, which tend to promote innate and adaptive immune responses, and the T-regulatory (Treg) cells, which usually suppress the inflammation resulting from innate and adaptive immunity. CD8 T cells are composed of the following two subgroups: cytotoxic T cells (Tc), which are the main cell killer in adaptive immunity, and CD8 Treg cells, which inhibit the activity of Th cells and suppress immune responses to infection. γδ-T cells participate in both innate and adaptive immunity. Their function involves not only immune effector pathways, that is, phagocytosis and tumor killing, but also immune regulation, as exemplified by the suppression of CD8 T cells (Table 1). In adaptive immunity in the liver, these T-cell subsets are highly orchestrated in terms of their roles and specific functions at each stage of various disorders.[4,6] To illustrate this, we will examine several representative hepatic diseases including infections, neoplasms and AILDs.

ADAPTIVE IMMUNITY IN HEPATIC VIRAL INFECTIONS

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are two of the most common chronic liver infections, which often lead to hepatic cirrhosis and HCC. In the United States, the prevalence of HBV and HCV infections is 4.9 and 1.8%, respectively.[6] Although HBV and HCV are both hepatotropic viruses, the real 'culprit' that accounts for the hepatocellular necrosis in HBV or HCV infection is primarily the adaptive immune response to virus-infected liver cells.[7]

Naive T cells that are specific for viral antigens can be activated locally in the liver. Conventionally, at the initiation stage of adaptive immunity, antigen-specific naive T cells are usually primed by antigen-presenting cells (APCs) in the lymph nodes and other lymphoid organs, where they proliferate, differentiate into effector cells and then migrate to the target site (such as the liver) to execute their effector functions.[8] However, HBV-specific naive T cells can directly enter the liver, be primed and exhibit the anti-HBV effects.[9,10] The liver also houses abundant APCs, such as myeloid DCs, plasmacytoid DCs, Kupffer cells, liver sinusoidal endothelial cells, hepatocytes and stellate cells.[11,12] In addition, activated platelets may promote the recruitment of HBV-specific CD8 T cells into liver, which can result in hepatic impairment of cytotoxic T cells, which normally clear the virus.[13]

T-cell responses in HBV infection

CD4 T cells in HBV infection. Different subsets of CD4 T cells have various immunopathological roles during HBV infection. In patients with HBV, the number of Th17 cells is significantly increased in the blood and liver, and is accompanied by elevated levels of interleukin (IL)-17 and IL-22 in the blood.[14] The roles of T cells and T-cell subsets have been investigated in animal models and clinical samples from patients with HBV. Adoptive transfer of spleen cells from HBV-immunized mice into HBV transgenic (Tg) mice exacerbates hepatic damage. However, the damage is markedly decreased by IL-22 inhibition. Because IL-22 is primarily produced by Th17 cells, these data support the hypothesis that Th17 cells have the potential to exacerbate liver lesions during HBV infection.[15] The immunomodulatory effects of CD4^{+}

Table 1 T-cell subsets and their functions in adaptive immunity

Cell group	Subset	Function	Reference
CD4 T cells	Th1	Secretion of IFN-γ, IL-2, TNF-α; activation of CD8 T cells; induction of Ig class switching to complement-fixing antibodies; cell-mediated and delayed-type hypersensitivity responses	186,187
	Th2	Secretion of IL-4, IL-5, IL-9, IL-10, IL-13 and IL-25; induction of Ig class switching to IgG1 and IgE; assisting in antibody-dependent cell-mediated cytotoxicity; association with allergic responses; suppression of Th1 cells and Th17 cells	186,188
	Th17	Secretion of cytokines IL-17, IL-21, IL-22, IFN-γ; suppression of Treg cell function	15,42,167,187,189,190
	Tfh	B-cell proliferation, differentiation and maturation in lymphatic tissue; antibody production; immune reaction; activation of CD8 T cells	139,163,191,192
	Treg	Secretion of IL-10 and TGF-β; inhibition of Th17 proliferation and secretion of IL-17; suppression of proliferation of CD4 CD25(-)T, CD8 T cells and their secretion of IFN-γ; suppression of innate immunity	165,173,193,194
CD8 T cells	Tc	Secretion of granzymes, perforin, IFN-γ, TNF-α, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17 and IL-21; cytotoxicity against tumors and intracellular pathogens; promotion of Th2-mediated allergy; propagation of autoimmunity	195,196
	CD8 Treg	Secretion of granzymes, perforin, TGF-β and IL-10; suppression of proliferation of Th cells; suppression of immunity in response to infection	54,197,198
γδ-T cells	γδ-T cell	Secretion of IL-10, IL-17, IL-22, TNF-α and IFN-γ; suppression of CD8 T cells; phagocytosis of bacteria; cytotoxicity against hepatic tumors; induction of hepatocyte apoptosis; limited hepatic inflammation and fibrosis	178,199–205

Abbreviations: IFN-γ, interferon γ; Ig, immunoglobulin; IL, interleukin; Tc, cytotoxic T cell; Tfh, follicular helper T cell; TGF-β, transforming growth factor-β; Th, helper T cell; TNF-α, tumor necrosis factor α; Treg, regulatory T cell.

CD25$^+$ Tregs on HBV have received significant attention. Several studies have demonstrated an immunosuppressive effect of HBV-specific CD4$^+$CD25$^+$foxp3$^+$ Treg cells during HBV infection. For example, such cells from chronic HBV patients suppress HBV-mediated interferon-γ (IFN-γ) production and the proliferation of autologous peripheral blood mononuclear cells.[16] In addition, the number of CD4$^+$CD25$^+$ Foxp3$^+$ Treg cells is increased in the peripheral blood and liver from HBV-associated acute-on-chronic liver failure and inhibits the proliferation of autologous CD4$^+$CD25$^-$ Th cells *in vitro*.[17]

CD8 T cells in HBV infection. Accumulating evidence demonstrates that HBV-specific CD8 T cells have a fundamental role in viral clearance and in the prognosis of HBV infection. Thimme et al.[18] have compared the consequences of CD4 T deletion with CD8 T deletion in HBV-infected chimpanzees and have demonstrated that the CD8 T cells are responsible for both noncytolytic and cytolytic anti-HBV functions in acute hepatitis B. In the noncytolytic process, naive CD8 T cells (primed in the liver) or activated CD8 T cells (primed in the extrahepatic lymphatic tissues) migrate into the liver. Once in the liver, the HBV-specific CD8 T cells and HBV-nonspecific innate immunocytes (macrophages) become activated and produce IFN-γ and tumor necrosis factor-α (TNF-α), which, in turn, noncytolytically inhibit HBV replication in infected hepatocytes and lead to viral clearance. The possible mechanisms for this inhibition and clearance include suppressing the assembly of the pregenomic RNA-containing capsids or accelerating their degradation, preventing the formation of the replication-competent HBV capsid and promoting proteasome-dependent HBV degradation.[19–21] However, the cytolytic anti-HBV function is achieved by the cytotoxicity of CD8 T cells. Furthermore, the data from experiments in HBV Tg mice have indicated that the noncytolytic and cytolytic antiviral actions of CD8 T cells are not only independent of each other but also occur in an asynchronous and oscillatory manner.[7,22] After the acute phase of resolving the HBV infection, some of the HBV-specific CD8 T cells differentiate into memory CD8 T cells with increased expression of CD127, which prevent future HBV reinfection.[23] However, in chronic HBV infection, which is the most common infectious state of this virus, the HBV-specific CD8 T cells are highly exhausted, displaying functional inhibition and even apoptosis.[24]

Possible factors for CD8 T-cell immune tolerance to HBV. On the basis of the functional changes in the CD4 and CD8 T cells during chronic HBV infection, it is logical to assume that restoring the functions of CD8 T cells from the tolerant or exhausted state may lead to the clearance of chronic HBV or HCV infection. Many studies have focused on the causes of tolerance in CD8 T cells in HBV infection. Studies on HBc/HBeAg Tg mice crossed with T-cell receptor (TCR) Tg mice, which express the receptors for the HBc/HBeAgs, have found that the HBV components induce specific immune tolerance through clonal deletion, clonal ignorance and clonal anergy.[25] The exhausted HBV-specific CD8 T cells from chronic

hepatitis B (CHB) can be functionally improved when the HBV loads are markedly reduced through treatment with viral suppressors such as Lamivudine.[26,27]

It has also been reported that there are more CD11b$^+$Gr-1$^+$ myeloid-derived suppressor cells (MDSCs) in the livers from patients with CHB. It is possible that the suppressive role of MDSCs on T cells may contribute to the dysfunction of HBV-specific CD8 T cells. γδ-T cells may promote CD8 T-cell exhaustion by recruiting the MDSCs to aggregate in the liver.[28,29] Programmed death-1 (PD-1) has been found to be highly expressed on the exhausted HBV-specific CD8 T cells from patients with CHB. Its expression is much stronger on the intrahepatic T cells than their peripheral counterparts and is one of the contributing factors to T-cell hypofunction because PD-1 inhibition restores T-cell effector functions, including proliferation, IFN-γ secretion and cytolytic activity.[24,30–33] This PD-1-mediated HBV-specific CD8 T-cell exhaustion can also be rescued by CD40-induced myeloid DCs.[10] PD-1 and the signaling lymphocyte activation molecule-related receptor (CD244) are highly co-expressed on HBV-specific CD8 T cells, but transmit their signals independently. CD244 inhibition with antibodies against either CD244 or its ligand CD48 restores the proliferation and cytotoxicity of the exhausted CD8 T cells.[31]

A higher percentage of HBV-specific CD8 T cells from patients with CHB express T-cell immunoglobulin (Ig) and mucin-domain-containing molecule-3 (Tim-3). These T cells produce abnormal levels of IFN-γ and TNF-α. Moreover, these patients have a higher concentration of galectin-9 (Gal-9), the ligand of Tim-3, in the sera and on the Kupffer cells in liver, thereby suggesting that their T cells are prone to Gal-9-triggered apoptosis and that the Kupffer cells might have a role in inducing immune tolerance, despite their classical role as APCs. Blocking Tim-3 with a recombinant Tim-3-Fc chimera revives the HBV-specific CD8 T-cell functions *ex vivo*.[34] Instead of binding to Gal-9, the HBV-specific Tim-3$^+$ CD8 T cells can also bind high-mobility group box 1 and act as virus-specific Treg cells, thereby downregulating the immune reaction against HBV.[35] Other inhibitory receptors on the HBV-specific CD8 T cells that might contribute to the exhaustion of T cells include B-cell lymphoma-2-interacting mediator (Bim) and cytotoxic T-lymphocyte antigen-4 (CTLA-4). Both may be co-expressed and have been reported to be upregulated on the CD8 T cells from patients with CHB. Therefore, inhibition of Bim and CTLA-4 also results in the recovery of T-cell functions.[36,37]

T-cell responses in HCV infection

CD4 T cells in HCV infection. The participation of CD4 T cells in adaptive immunity against HCV varies during the acute and chronic phases of infection, which are associated with the initiation, recovery or persistence of the infection.

In acute resolving HCV infection, there are more HCV-specific CD4 T cells, with higher immune activity.[38,39] Among the cytokines secreted from the CD4 Th1 cells, IL-2 is vital for CD4 and CD8 T-cell activation. The function of the CD8

T cells decreases markedly, with respect to IFN-γ production and proliferation, when the CD4 T cells lose their ability to produce IL-2 during the chronic stage of HCV infection. Although HCV-specific CD8 T cells have the capability to proliferate, exhibit cytotoxicity and secrete IFN-γ, they are not sufficient to clear the HCV. Priming from HCV-specific CD4 T cells and the presence of IL-2 are critical in determining the outcome of the infection.[40,41] CD4 Th17 cells secrete IL-17 and IL-21. Patients with higher plasma concentrations of IL-17A and IL-21 in the acute phase are predisposed to a self-limited infection. They also have more CD4 T cells that secrete IL-21 than do patients with a chronically evolving HCV infection. Kared et al.[42] has shown that the IL-21 from the CD4 T cells promotes the expansion of HCV-specific CD8 T cells and prevents their Gal-9-induced apoptosis in vitro, even though they express inhibitory receptors (Tim-3). In addition, in acute resolving hepatitis C, the CD4 T cells contribute to the maturation of memory CD8 T cells, which prevent reinfection with HCV.[43] When the CD4 T cells in HCV-immunized chimpanzees are depleted, HCV reinfection results in incomplete control of the viral replication, persistent viremia and viral mutations that escape the adaptive immune system, even though the CD8 T cells in liver respond with normal function.[39]

In contrast, the CD4 T cells in chronically evolving hepatitis C exhibit different phenotypes, a high-level expression of inhibitory receptors (Tim-3, PD-1 and CTLA-4) accompanied by a decreased frequency of IL-21-secreting CD4 T cells[42,44] and an increased number of CD4+CD25+Foxp3+ Treg cells, which then directly suppress the HCV-specific CD8 T cells in vitro.[45,46] Moreover, these cells perform their immunosuppressive functions by altering the innate immune system.[47]

CD8 T cells in HCV infection. By producing cytokines (mainly IFN-γ) and inducing cytotoxicity as their major antiviral mechanisms, CD8 T cells are responsible for the hepatic lesions in HCV, as well as the viral cytopathic effects. Thus, serum hepatic enzyme levels change synchronously with the frequency of the HCV-specific CD8 T cells but not with the HCV viral load.[48] HCV-specific CD8 T cells have a fundamental role in long-term protection against HCV. Although HCV-immunized chimpanzees produced HCV-specific CD4 T cells, the depletion of CD8 T cells reduces the suppression of HCV replication.[49] Unfortunately, extensive data have also indicated that the HCV-specific CD8 T cells are impaired or exhausted in chronic HCV infections. It has also been noted that the number of intrahepatic and cycling HCV-specific CD8 T cells does not decrease in the chronic phase, and their capacity for proliferation, IFN-γ secretion and cytotoxicity are all significantly impaired, thus limiting their efficacy in HCV clearance.[50–52]

Possible factors for CD8 T-cell tolerance to HCV. Given that the immune tolerance of CD8 T cells is one of the major causes of chronic HCV infection, it is necessary to discern the possible factors that regulate tolerance. A variety of inhibitory mechanisms are known to be associated with the immune tolerance of the HCV-specific CD8 T cells in chronic HCV infection. First,

HCV itself is likely to impair the function of these T cells. When HCV is efficiently suppressed by HCV inhibitors, the frequency and proliferation of HCV-specific CD8 T cells is markedly increased, but this increase does not appear in those who did not respond to the same treatment.[53] In addition, after repeat immunization of chimpanzees with a low dose of HCV, reinfection with HCV reduces IFN-γ secretion from the HCV-specific CD8 T cells in the liver.[38] These studies have demonstrated that the HCV load itself can affect the function of the HCV-specific CD8 T cells. Second, aside from the suppressive modulation from the CD4+ Tregs mentioned above,[45,46] the HBV-specific CD8+ Tregs also exhibit an inhibitory effect on the CD8 T cells by secreting the suppressive cytokine IL-10.[54] Third, the HCV-specific CD8 T cells in liver and blood express PD-1 during both the acute and chronic phases of HCV infection; this expression disappears in the recovery phase, but remains at high levels in persistent infections. In the chronic stage, PD-1 expression is associated with impaired function of HCV-specific CD8 T cells, and PD-1 inhibition reactivates CD8 T-cell functions in vitro, including proliferation, IFN-γ production and cytotoxicity, which are responsible for HCV inhibition and clearance.[55–58] Fourth, the expression level of another inhibitory receptor, Tim-3, on the HCV-specific CD8 T cells is correlated with CD8 T-cell exhaustion and blocks the interaction between Tim-3 and Gal-9, thus restoring the CD8 T cells' immune functions.[42,59,60] Finally, it has been reported that the inhibitory receptor CTLA-4 is preferentially upregulated in PD-1+ T cells in the livers of chronically HCV-infected patients, which also contributes to HCV-specific T-cell exhaustion. Inhibitors that block both the CTLA-4 and PD-1 inhibitory receptors exhibit a synergistic effect against chronic HCV infection by reactivating the HCV-specific CD8 T cells.[61]

In addition to inducing immune tolerance, HCV can escape adaptive immunity through mutations,[62] which mainly accounts for the failure to produce an HCV vaccine.

Together, these findings indicate that cellular immunity, particularly the adaptive immunity mediated by CD8 T cells, has a critical role in the inhibition and clearance of HBV and HCV. This adaptive immunity results in inflammatory damage to hepatocytes. CD8 T-cell immune tolerance to the virus might be beneficial for preventing hepatic injury but would be conducive to persistent infections, which could increase the risk of hepatic cirrhosis and hepatic cancer after prolonged chronic infection. Therefore, therapeutic interventions designed to disrupt T-cell immune tolerance to HBV or HCV may be a double-edged sword in terms of their effects on the immunopathogenesis of these chronic infections. Figure 1 illustrates the functional changes in the CD8 T cells and their effects on chronic HBV or HCV infections (Figure 1). Although it is still unclear whether immune tolerance is caused by viral factors to promote their survival or by host factors to limit the hepatic lesions, previous studies have identified potential targets that disrupt the immune tolerance and restore the immune function of exhausted virus-specific CD8 T cells, such as blocking the inhibitory receptors on T cells and reducing the HBV/HCV
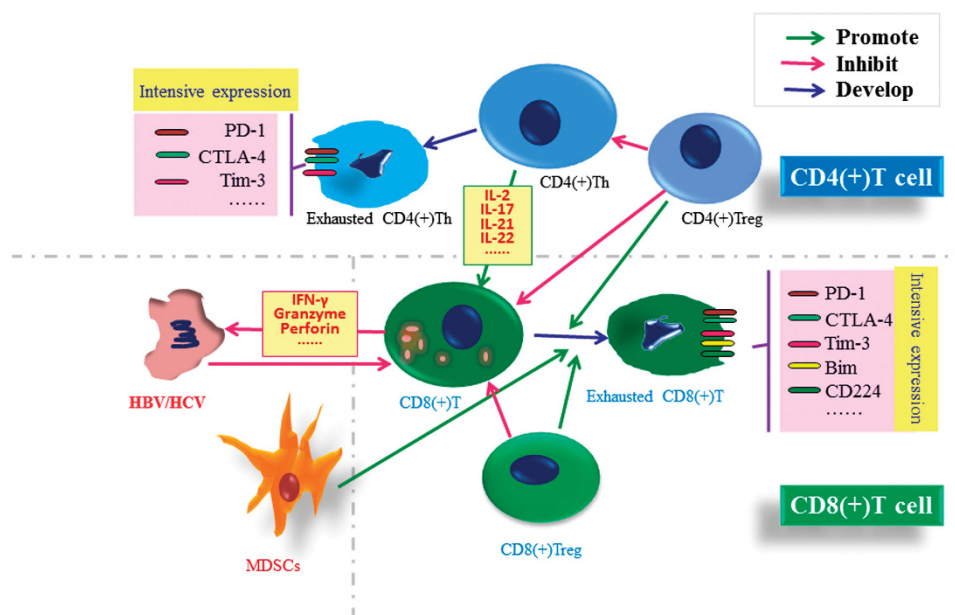
Figure 1 A schematic diagram of the functional changes and some of the influential factors in chronic HBV/HCV infections. The ability of CD8 T cells (Tc) to clear HBV/HCV is inhibited by various factors. HBV and HCV are inhibited by the virus-specific Tc. Both CD4 Treg and CD8 Treg cells suppress Tc function and promote the switch of the Tc cells from the active state in acute phase to the exhausted state in the chronic phase. CD4 Th cells are indispensable for the activation and maintenance of Tc immunity against HBV/HCV. However, Th cells can also be suppressed and exhausted similarly to Tc cells. MDSCs are another factor that facilitates Tc exhaustion. Increased expression of inhibitory protein receptors (such as PD-1, CTLA-4, Tim-3, Bim and CD224) on Tc/Th cells contributes to their exhaustion. Therefore, treatments that block the interaction between these receptors and their ligands, and inhibit Treg functions are potential therapeutic strategies for chronic HBV/HCV infections.

viral loads with various drugs. Inhibiting the Treg cells could also be a potential therapeutic strategy for chronic HBV or HCV infections.

ADAPTIVE IMMUNITY TO HCC

HCC is the most common primary malignant tumor in the liver and the fifth-most common neoplasm worldwide, representing the third-most common cancer-related death. Many cases of HCC occur in subjects with a history of chronic liver diseases, such as HBV or HCV infection, PBC and hepatic cirrhosis. At least two possible mechanisms—immunosuppression due to viral infection and viral gene integration—account for the close association between viral infection and HCC.[63–65] The occurrence and prognosis of HCC are closely related to immunity, particularly T-cell-mediated adaptive immunity. Studies on lymphocytes in livers from patients with HCC have demonstrated reduced lymphocyte infiltration, which is a significant, independent predictor for relapse. These lymphocytes display different phenotypes and functions when located in the intratumoral and peritumoral regions.[66,67] Because most HCC patients cannot tolerate the toxicity of traditional chemical anticarcinogens, immunotherapy is an alternative approach.

CD8 T cells in HCC

It is generally accepted that CD8 T cells are the fundamental adaptive immunocytes that monitor and kill tumor cells via histocompatibility leukocyte antigen class I molecule (HLA-I)

limitation on the tumor cells. Over the past two decades, several HCC tumor-associated antigen (TAA)-specific CD8 T cells have been identified. Alpha-fetoprotein (AFP) is the most common TAA in HCC. Its epitopes are recognized by specific CD8 T cells and are broadly distributed on the AFP polypeptide, suggesting a strong and broad immunogenicity of this TAA.[68] It has been reported that AFP converts DCs into tolerogenic DCs, which subsequently inhibit the induction of tumor-specific CD8 T cells and suppress their cytotoxic activity.[69] Other TAAs that are recognized by specific CD8 T cells in HCC include telomerase reverse transcriptase,[70] the targeting protein for Xklp-2,[71] glypican-3 (GPC3),[72] NY-ESO-1,[73] melanoma antigen gene-A and SSX-2.[74] These TAAs are detected in other cancers but are often overexpressed in HCC cells, with some of them contributing to the growth and metastasis of HCC.[72,73] However, TAA-specific CD8 T cells lose their ability to effectively suppress or kill malignant hepatic cells in individuals with HCC. Notably, accumulating evidence indicates that suppressive CD8$^+$ Tregs also increase in HCC. These tumor-infiltrating CD8$^+$FoxP3$^+$ Tregs may contribute to HCC immune evasion and disease progression.[75]

Possible factors affecting CD8 T-cell immunity in HCC

There is no doubt that the identification of the causes of the immune tolerance is of critical importance in developing immunotherapies for HCC, which are aimed at improving CD8 T-cell function. Previous studies have demonstrated that

there are multifaceted underlying causes for the CD8 T-cell functional suppression.

CD4 T cells in HCC. CD4 Th cells are essential for the CD8 T-cell response, but Th cells are reduced in HCC. TAA-specific CD4 Th cells are detected in early stages of HCC but are exhausted in advanced HCC, although they are still detected in the circulation and in hepatic tumors at lower frequencies.[76,77] The exhausted CD4 Th cells may be reactivated to produce IFN-γ if their inhibitors are removed.[78]

Among the CD4 T-cell subsets in HCC, CD4$^+$CD25$^+$Foxp3$^+$ Treg cells have the most important immunoregulatory role. Marked infiltration of Treg cells has been observed in the livers from patients with HCC, and the number of intratumor Treg cells is increased compared with the peritumor regions and periphery. They are also associated with tumor vascular invasion. However, the number of CD8 T cells in the liver decreases as the number of infiltrating Treg cells increases. Their proliferation and perforin production are also suppressed by the autologous Treg cells isolated from patients with HCC.[66,79,80] These isolated Treg cells effectively suppress the proliferation and cytokine secretion of CD4$^+$ CD25$^-$ T cells.[80] Moreover, when the number of Treg cells from patients with HCC is decreased by a low-dose cyclophosphamide treatment, the number of IFN-γ-secreting AFP-specific CD4 T cells subsequently increases.[81] These studies have indicated that, in addition to the direct suppression of the CD8 T cells, the CD4$^+$CD25$^+$Foxp3$^+$ Treg cells indirectly suppress CD8 T cells by inhibiting CD4 T cells. Furthermore, the secretion of inhibitory cytokines (IL-10, TGF-β and so on) is one of the major immunosuppressive mechanisms of Treg cells.[82] A recent report has indicated that TGF-β promotes Treg differentiation in an HCC mouse model, which contributes to the progression of HCC.[83] This implies that Treg activation produces a positive, immunosuppressive feedback loop in HCC.

MDSCs and other factors that influence the CD8 cells in HCC. In addition to CD4 T cells, other factors are involved in the immune tolerance to HCC (Figure 2). It has been found that MDSC infiltration is markedly increased in HCC. Some studies have also found that the number of MDSCs is increased in the peripheral lymphatic tissue and blood, resulting in extensive suppression of both innate and adaptive immunity.[84–86] MDSCs suppress natural killer cells in HCC by cell-cell contact.[87] Although there is no evidence for direct MDSC-mediated suppression of TAA-specific CD8 T cells in HCC, the results from several studies have suggested that MDSCs inhibit CD8 T cells through indirect pathways. MDSCs produce inhibitory cytokines, such as IL-10, thereby suppressing CD8 T cells because HCC patients with increased numbers of MDSCs also have higher levels of IL-10.[86] MDSCs also indirectly impair the functions of CD8 T cells through autologous CD4$^+$CD25$^+$Foxp3$^+$ Treg cells because the latter can be activated when co-cultured with MDSCs.[88] In addition, the suppression of DCs by MDSCs might inhibit adaptive immunity in HCC by reducing TAA presentation on the DCs.[86]
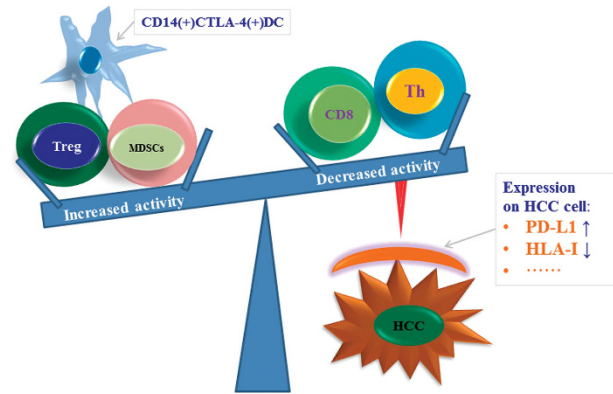

Figure 2 Changes in the functions of the major adaptive immune cells in HCC. CD8 T cells, the primary HCC-specific killer and its helper CD4 CD25$^-$ Th cells are inhibited, whereas the inhibitory immune cells, including Treg cells, MDSCs and CD14$^+$CTLA-4$^+$ DCs, are increased in HCC patients. Moreover, the HCC cell itself can evade adaptive immune surveillance through several strategies, such as reduced expression of the HLA-I antigen and enhanced expression of PD-L1, the ligand of PD-1, which is expressed on the tumor-filtrating T cells and mediates T-cell suppression or apoptosis. These functional alterations of the T cells promote HCC occurrence and development.

Recently, CD14$^+$ CTLA-4$^+$ DCs have been detected in patients with HCC. These DCs are thought to be a new subset of DCs because they secrete the immunosuppressive cytokine IL-10 and indoleamine-2, 3-dioxygenase, which are dependent on CTLA-4 expression. The *in vitro* suppressive effect of DCs on CD4 T cells has also been experimentally verified.[89] These regulatory DCs inhibit CD8 T cells via both immunosuppressive cytokines and downregulation of CD4 T cells.

As discussed above in the section on adaptive immunity to viral infection, PD-1 is a well-known immunosuppressive receptor on T cells. It has been shown that PD-1 is highly expressed on T cells that are infiltrating the hepatic tumor and in the circulation, whereas PD-L1, the ligand of PD-1, is overexpressed on hepatic tumor cells.[90–94] *In vitro*, the IFN-γ secreted by CD8 T cells with increased PD-1 expression induces high levels of PD-L1 expression on cancer cells,[91,92] which may lead to exhaustion of the TAA-specific CD8 T cells in the tumor through a vicious feedback loop and subsequent tumor cell immune escape. Thus, the increased PD-L1 expression on HCC cells might have valid prognostic value in that its expression levels are inversely related to HCC prognosis.[93,95]

For HCC developed from HBV/HCV infection, all of the aforementioned immunosuppressive mechanisms related to the viral infections might also contribute to the immunosuppression in HCC. To escape from the host's immune surveillance, HCC cells adopt various mechanisms, including reduced HLA-I antigen expression to elude CD8 T-cell attack[95,96] and enhanced PD-L1 expression to inhibit CD8 T cells with high levels of PD-1 expression.[91] Unfortunately, the underlying escape mechanism remains unclear, which could account for the poor efficacy of most immunotherapies currently available for HCC, even though these immune interventions have been

validated in *ex vivo* experiments. Additional studies on HCC adaptive immunity are necessary.

ADAPTIVE IMMUNITY IN AILDS

AILDs are mainly composed of PBC, primary sclerosing cholangitis and AIH, among which PBC and AIH will be the focus of this review. Unlike viral hepatitis and HCC, in which the adaptive immune system targets the virus-infected cells and cancer cells, the adaptive immune system targets normal hepatic parenchymal cells (biliary ductule cells and hepatocytes) in AILDs, although most patients with chronic viral hepatitis infections and AILDs eventually present hepatic cirrhosis and even liver cancer.

Adaptive immunity in PBC

PBC is one of the most common autoimmune hepatic diseases. Although they vary among regions and races, PBC prevalence and incidence have increased in recent decades.[97,98] PBC is a typical organ-specific autoimmune disease, in which the biliary ductule is the major target of destruction. Patients with PBC suffer from symptoms ranging from lymphocytic cholangitis to progressive ductopenia, which are associated with cholestasis and biliary fibrosis.[99,100] Recent studies in patients and animal models have demonstrated that the interplay of genetics and the environment with the innate and adaptive immune systems is highly orchestrated in the pathogenesis of PBC.[101–113] The presence of antimitochondrial antibodies (AMA), particularly the autoantibody against pyruvate decarboxylase E2 (PDC-E2), is the serological hallmark of PBC and has a potential pathogenic role.[114–116] However, liver-infiltrating autoreactive T lymphocytes also have crucial roles in the destruction of the small bile ducts.

CD8 T cells in PBC. Among the T-cell subsets, CD8 T cells have a decisive role in the immunopathogenesis of PBC. In PBC patients, CD8 T cells abundantly infiltrate the hepatic portal regions. Whereas PDC-E2-specific CD8 T cells are detected in the peripheral blood at early stages of PBC, their frequency in the liver-infiltrating lymphocytes is 10 times higher than that in the blood.[117,118] In experimental mouse models of PBC, the liver lesions are accompanied by extensive CD8 T-cell infiltration in the portal region, granuloma and even fibrosis.[119–124] In addition, these animals exhibit increased serum levels of AMA, TNF-α and IFN-γ. Importantly, the significance of the CD8 T cells in PBC is illustrated by the induction of PBC with adoptive transfer of CD8 T cells, but not CD4 T cells, from the dnTGF-βRII mouse model of PBC to recipient C57BL/6J mice.[122,125] Furthermore, instead of the extrinsic factors around the CD8 T-cell environment, the intrinsic deficiency (abnormal TGF-βRII signaling) in CD8 T cells *per se* determines that the cholangiocytes are the target of the transferred CD8 T cells.[126] This crucial role of CD8 T cells partially explains the pathogenesis in AMA-negative PBC patients.

CD4 T cells in PBC. The autoimmune pathogenesis in PBC is also orchestrated by different subsets of CD4 T cells.

Infiltration of CD4 T cells, including major histocompatibility complex class II-restricted PDC-E2-specific CD4 T cells, is evident in the inflammatory portal area in the livers from PBC patients or animal models.[123,127–129] PDC-E2-specific CD4 T cells have also been observed in AMA-negative PBC patients.[129] In PBC patients and mouse models of PBC, increased numbers of CD4 T cells (Th17) have been observed within the portal tracts compared with the periphery.[130] An analysis of IL-12/Th1 and IL-23/Th17 biliary microenvironment has suggested that the Th17 cells have a dominant role in the perpetuation of PBC immunopathology mediated by the Th1 cells at early stage.[131]

The T-cell populations, including Treg cells and Tfh cells, have also recently been examined in PBC. Unlike the Treg cells in HCC, those in PBC display a decreased immunosuppressive function. The frequency of CD4+CD25+Foxp3+ Treg cells is reduced in the blood and inflammatory portal tracts of patients with PBC. The ratio of CD8 T cells/Treg cells increases with disease progression.[132] Similar findings have been reported in Ae2$_{ab}$$^{-/-}$, foxp3$^{sf/Y}$ Sf[123] and IL-2 receptor knockout (IL-2R$^{-/-}$) mouse models of PBC.[125] Interestingly, a case report has described a subject with a classical PBC clinical manifestation (positive for the PDC-E2 antibody and PBC hepatic histopathology) due to a congenital deficiency of the alpha subunit of the IL-2 receptor.[133] In the absence of Treg cells from the donor dnTGF-βRII mice, it is difficult to induce PBC in the recipient mice solely by adoptive transfer of CD8 T cells. Normal, functional Treg cells (such as those from B6 mice) reduce hepatic damage in the recipient mice, which is associated with reduced T-cell cytotoxicity toward the cholangiocytes after CD8+ T-cell transfer.[134,135] In fact, a recent study on Treg cells from the dnTGF-βRII mice has confirmed that genetic defects in the TGF-β signaling pathway are responsible for the immune dysregulation and subsequent inflammatory upregulation in the PBC mouse model.[136] In another study, CD4$^{-/-}$ dnTGF-βRII Tg mice have been generated to examine the contribution of CD4 cells to the autoimmune cholangitis in the dnTGF-βRII mice. The CD4$^{-/-}$ dnTGF-βRII Tg mice produce more IFN-γ and exhibit a more severe biliary pathology than do the dnTGF-βRII mice. Interestingly, the liver pathology is alleviated by adding back the wild-type CD4+ T cells, which contain Tregs, through bone marrow transplantation or parabiosis.[137] These observations suggest that the Treg cells from wild-type mice modulate biliary disease by limiting the differentiation of autoantigen-specific CD8+ T cells. In summary, these studies have suggested that Treg cells might be a potential target of immunotherapy for PBC. However, PDC-E2-specific Treg cells have yet to be identified. In patients with PBC, increased numbers of Tfh cells, which are associated with elevated levels of IL-21, have been observed in the blood, liver and spleen. However, the increased numbers of Tfh cells might decrease significantly following effective ursodeoxycholic acid treatment.[138,139]

Other possible factors affecting T-cell immunity in PBC. T-cell function in PBC is also influenced by other factors, including

cholangiocytes and various receptors on T cells. In the immunopathological process of PBC, cholangiocytes not only are the target and victim of immune reaction but also are active factors in the immune response (Figure 3). Cholangiocytes express the HLA-II antigen and act as APCs. CD80 and CD86 costimulators are expressed on cholangiocytes and interact with the CD28 receptor on T cells, resulting in T-cell activation. Cholangiocytes also facilitate innate and adaptive immunity by producing cytokines and ILs, such as IL-6, IL-8 and monocyte chemotactic protein-1 (MCP-1).[140] By expressing the adhesion molecule CX3CL1 (chemokine-adhesion molecule (fractalkine)), cholangiocytes recruit CD4 and CD8 T cells that express CX3CR1 (adhesion molecule, the ligand of CX3CL1).[141] CD40 on cholangiocytes induces cholangiocyte apoptosis and activates macrophages after binding to the CD40 ligand (CD40L) on macrophages.[142] It is likely that the CD40L expressed on T cells has a similar function. Cholangiocyte-specific epitopes on immunologically intact PDC-E2 might be one of the mechanisms underlying the biliary-specific autoimmune pathogenesis in PBC.[143]

A study on genetic and epigenetic modifications of the CD40L gene in circulating CD4 T cells from PBC patients and healthy controls has found that the degree of DNA methylation of the CD40L promoter is lower in PBC patients than controls, which might lead to increased CD40L expression on CD4 T cells and promote their activity. Furthermore, this study has also demonstrated that the decrease in methylation is inversely related to the serum levels of IgM.[144] Nevertheless, a preliminary study has shown that blocking CD40L with an anti-CD40L antibody has limited therapeutic effects in a mouse model of PBC (dnTGF-βRII Tg mice).[145] In another study using a 2-octynoic acid-conjugated bovine serum albumin (2OA-BSA)-immunized mouse model of PBC to examine the therapeutic effect of CTLA-4-Ig, which inhibits CTLA-4, CTLA-4-Ig has been found to prevent disease onset when administered before the 2OA-BSA immunization. Even after the appearance of liver lesions, the administration of CTLA4-Ig reduces intrahepatic T-cell infiltrates and biliary cell damage.[146] These results indicate that CTLA-4-Ig might have a potential therapeutic value for PBC.

It should be noted that some patients with PBC have an increased risk for hepatic carcinoma or present extrahepatic malignancies and other autoimmune conditions.[147–150] Tools in immunogenetics, molecular biology, immunopathology, bioinformatics and systems analysis will continue to facilitate the unveiling of the underlying adaptive immune mechanisms and the identification of the relevant clinical markers in monitoring the prognosis of PBC, and as possible clinical interventions for the T-cell responses in PBC.[151–155]

Adaptive immunity in AIH

AIH is a common autoimmune chronic inflammatory liver disease characterized by the presence of multiple autoantibodies, elevated serum aminotransferase levels, excessive hepatic lymphoplasmocytic infiltration and interface hepatitis. Its prevalence varies in different geographical areas and races. In spite of the currently available effective therapeutic agents, such as corticoid and azathioprine, AIH accounts for 10–54% of cryptogenic cirrhosis.[156,157] The exact pathogenesis of AIH remains an enigma. However, accumulating data indicate that T cells have pivotal roles in the immunopathogenesis of AIH through immune regulation and immune effector functions, and multiple autoantibodies are also important participants.[158,159]
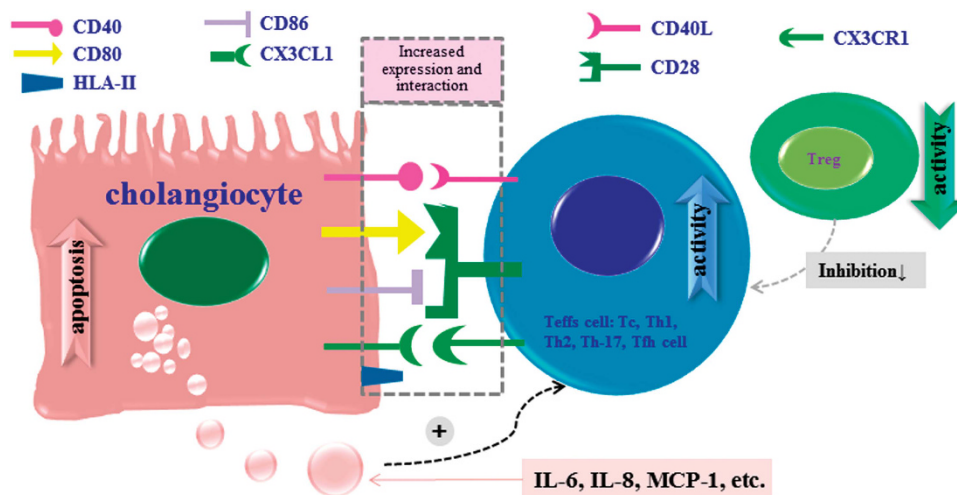

Figure 3 A schematic representation of the interaction between adaptive immunocytes and cholangiocytes in PBC. Teffs, which include Th1, Th2, Th17 and Tfh cells, are, at least in part, over-activated by downregulated Treg cells. Cholangiocytes are both the victim and accomplice of the autoimmunity in PBC. Upon aberrant expression of HLA-II, cholangiocytes can act as APCs to promote adaptive autoimmunity in the liver. The expression of CX3CL1 contributes to recruiting T cells to the liver. CD40, CD80 and CD86 expressed on cholangiocytes activate Teffs. Furthermore, the cytokines and interleukins (such as IL-6, IL-8 and MCP-1) secreted by the cholangiocytes promote autoimmunity. Collectively, these factors will increase adaptive autoimmunity and cholangiocyte apoptosis in PBC.

CD8 T cells in AIH. In AIH patients, the ratio of liver CD8/CD4 T cells increases with the development of disease activity.[160] A hepatic pathological study has indicated that emperipolesis is one of the characteristics of AIH, as observed in 65.3% of livers from AIH patients, whereas the percentage was only 17.9, 14.9 and 25.6% in patients with PBC, HBV infections or drug-induced liver injury, respectively. This emperipolesis is predominantly mediated by CD8 T cells and is correlated with higher serum aminotransferase (alanine transarninase/aspartate aminotransferase) levels and severe necroinflammation and fibrosis in the liver.[161] Evidence from animal studies has also supported the important roles of CD8 T cells in the pathogenesis of AIH. In AIH mouse models, CXCR3 and CCR6 are highly expressed on CD8 T cells, which facilitate their recruitment to the liver because their respective ligands, CXCL9 and CCL20, are highly expressed in inflamed liver.[162,163] Adoptive transfer of total splenocytes, but not CD4 T-cell-depleted splenocytes from an AIH model (NTx-PD-1$^{-/-}$ mice), into RAG2$^{-/-}$ recipient mice leads to massive inflammatory necrosis of the hepatic parenchyma. In contrast, the transfer of CD8 T-cell-depleted splenocytes in this AIH model alleviates hepatic damage.[164] In fact, the hepatic damage caused by CD8 T cells in AIH is at least partially owing to the dysregulation of other related innate and adaptive immune cells.

CD4 T cells and other related immunocytes in AIH. It has been found that different subsets of CD4 T cells, particularly Treg cells, exhibit notable effects in AIH. Histopathologically, the frequency of infiltrating CD4 T (Th) cells is higher than CD8 T cells at early stage AIH.[160] The spontaneous apoptosis of CD4 T cells is markedly reduced, and their Tim-3 expression is clearly increased in AIH.[165,166] Compared with those in the CHB patients and healthy controls, the frequencies of Th17 cells in both the liver and blood, and the circulating and intrahepatic levels of IL-17 are substantially elevated in AIH. This increased hepatic expression of IL-17 in AIH is associated with inflammation and fibrosis in liver.[167] In an adoptive transfer experiment with an AIH mouse model, the recipient mice (RAG2$^{-/-}$ mice) have been found not to develop AIH when the CD4 T cells are selectively deleted from the donor splenocytes from the NTx-PD-1$^{-/-}$ AIH model mice.[164] This finding shows that the CD4 T cells promote autoimmunity in AIH mice.

Treg cells have attracted much attention over the past decade because of their immunosuppressive functions and potential therapeutic value.[168] In animal experiments, neonatal PD-1-knockout mice spontaneously develop fatal AIH (mouse AIH model) when the thymus is excised to reduce the number of Treg cells. This AIH induction is suppressed by adoptive transfer of Treg cells.[164] Similar models have been used in other AIH studies.[162,163,169] In AIH patients, Treg cells suppress autoimmunity by direct contact with the CD4$^+$CD25$^-$ T cells and the secretion of regulatory cytokines such as IL-4, IL-10 and TGF-β.[165] In addition, the Treg cells might mediate immune suppression through the expression of CD39 and CD73. CD39 is an ectoenzyme (nucleoside triphosphate diphosphohydrolase-1, NTPDase-1) that degrades ATP to AMP. The decreased ATP levels reduce ATP-related effects, as exemplified by the P2 receptor-induced cytotoxicity and DC maturation. CD73 is an ecto-5′-nucleotidase that converts extracellular 5-AMP to adenosine. This adenosine might bind to the adenosine A2A receptors on activated T cells and exert immunosuppressive effects. Hence, the co-expression of CD39 and CD73 on Treg cells and their concerted effect are likely to be another immunosuppressive mechanism of Treg cells.[170–172] The Treg cells in AIH exhibit not only reduced expression of CD39 but also reduced NTPDase-1 activity, and also a reduced ability to inhibit IL-17 secretion from Th17 cells, which might contribute to autoimmunity in AIH.[173] The interaction between Gal-9 on the Treg cells and Tim-3 on the Th cells might be an important mechanism for the direct contact suppression mediated by Treg cells. Another study has demonstrated that AIH patients exhibit reduced levels of Gal-9 and Tim-3 on the Treg cells and CD4$^+$CD25$^-$ effector cells, respectively.[166] Although decreased numbers of Treg cells in AIH have been reported in a number of studies,[173–175] other studies have recently demonstrated that the Treg cells are not impaired in AIH.[160,176] These authors have found that neither the number nor the suppressive function of Treg cells in the liver and blood of AIH patients is reduced, as compared with healthy controls. The frequency of Treg cells in patients with active AIH is significantly higher than those in the remission stage. In addition, the intrahepatic ratio of Treg/Teffs (effector T cells) in patients who respond to treatment is higher than that in nonresponders. In *in vitro* experiments, Treg cells are more sensitive to the immune suppressor corticoids than T effective cells, which might partially explain the high relapse rates when the current immunosuppressive treatment is discontinued.[160,176] The intrahepatic microenvironment, including the cytokine and chemokine profiles, also affects the numerical and functional imbalance of the Treg and Teffs in AIH patients.[177] Together, these results suggest that the role of Treg cells in AIH immunopathology remains controversial.

In addition to Th and Treg cells, other immune cells are associated with adaptive cell immunity in AIH. A recent study in an AIH animal model has demonstrated that CD8 T cells are activated by IL-21 secreted by Tfh cells, which is surprising because Tfh cells are widely accepted as a special subset of CD4 T cells that assist with B-cell development.[163] γδ-T cells account for 15–25% of T cells in the liver. They not only act as cytotoxic T cells that regulate death receptor-mediated apoptosis and cytolytic degranulation but also act as immunoregulatory cells that secrete various cytokines, such as IFN-γ, IL-17, IL-4, IL-10, TGF-β and granulocyte-macrophage colony-stimulating factor (GM-CSF).[178] Indeed, the number of γδ-T cells in AIH patients is increased, and they secrete higher levels of IFN-γ and granzyme B than those in healthy controls, which might contribute to the autoimmune damage in AIH.[175] A recent study has demonstrated that B cells inhibit CD4 T cells in an AIH mouse model. This suppressive function is dependent on the expression of CD11b on B cells

and is mediated by impairing TCR signaling transduction and promoting TCR downregulation. IL-10 is mainly secreted by CD4 T cells and enhances CD11b expression, thus implying that CD4 T cells and B cells might regulate each other in AIH.[179]

SUMMARY

In conclusion, adaptive immunity, particularly cellular adaptive immunity, has a very important role in the immunopathology involved in the onset, development and prognosis of infection, tumor formation and autoimmunity in the liver. Adaptive immunity in the liver is mediated by numerous factors with extensive and complex interactions. In general, immunosuppression is dominant in persistent infections, such as chronic HBV or HCV infections, and hepatic tumors, such as HCC, whereas over-reactive immunity controls the pathogenesis in AILD, both of which can be ascribed to an immunoregulatory disorder.[17,180–185] The current limited understanding of this disorder has restricted the development of effective interventions to correct the decreased or increased adaptive immunity. Although this review primarily focuses on the current knowledge regarding the adaptive immunity associated with several representative diseases, adaptive immunity in the liver probably counters more than infections, neoplasms and hepatic autoimmune diseases. Moreover, the innate immunity in the liver is also very important in hepatic immunopathology, which is reviewed in several other papers in this issue. Innate and adaptive immunities interact and closely regulate each other in a complex manner through the process of hepatic immunity, thus making the regulation of adaptive immunity in liver more enigmatic. With the growing knowledge of adaptive liver immunology, physicians and scientists are much better equipped to further examine the multiple facets of human liver diseases and identify more effective clinical interventions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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