Chapter 4

The Role of Monocytes/Macrophages in HBV and HCV Infection

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Additional information is available at the end of the chapter

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Abstract

Monocytes/macrophages constitute the first line of defence for external intrusion or infection. Circulatory monocytes represent about 10% of leukocytes in human blood and resident macrophages are distributed in a variety of tissues and organs to maintain body homeostasis. But relatively little is known about the consequences of chronic viral infections on monocytes. Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are the most important causes of chronic liver diseases, which may develop to serious and fatal liver pathology, including liver cirrhosis and hepatocellular carcinoma. Whether HBV and HCV infections are cleared or persist is determined by host immune responses. Viral replication takes place inside hepatocytes as soon as infection begins. The secretion of infectious virions or virus proteins can persist for decades at high rates. Chronic infections with HBV and HCV are the result of ineffective anti-viral immune response towards the virus. Interacting with virions or virus proteins, monocytes/macrophages play an important function in the disease process. The role of monocytes/macrophages in HBV and HCV infections or co-infections is discussed in this chapter.

Keywords: monocytes, macrophages, hepatitis B virus, hepatitis C virus

1. Introduction

The significance of the innate immune response as a defence against microbial infections and its link to the adaptive immune responses have become increasingly recognized during the past few years. The activation of the innate immune response generally leads to the production of type I IFNs. Monocytes/macrophages constitute the first line of defence for external intrusion or infection. Circulatory monocytes represent about 10% of leukocytes in human blood and resident macrophages are distributed in a variety of tissues and organs to maintain

body homeostasis. But relatively little is known about the consequences of chronic viral infections on monocytes.

Hepatitis B virus (HBV) infection is a major health problem that affects around 350 million people worldwide, despite the availability of a prophylactic vaccine [1]. The number of the chronic HBV infection has already beyond 240 million because a great fraction of patients is unable to clear the virus spontaneously, although there remain a lot of patients clearing the HBV virus at the early stage [2]. Up to date, chronically infected patients are at high risk of developing HBV-related diseases such as liver cirrhosis and hepatocellular carcinoma, which account for 600,000 deaths annually [3]. The interaction between the HBV and an affective inadequate immune response could lead a chronicity of HBV infection [4–6]. Viral replication takes place inside hepatocytes as soon as infection begins. The secretion of infectious virions or virus proteins can persist for decades at high rates. HBV DNA, HBeAg and HBsAg can be easily detected in serum consequently. The levels of these clinical marker means HBV DNA,HBsAg for clinical diagnosis levels could fluctuate for a long time and keep sistency, are a reflection of virus duplicate activity and used to define the patients disease stage [5, 6].

Hepatitis C virus infection is considered as the most serious cause of chronic liver disease and hepatocellular carcinoma worldwide in the past 50 years [7, 8]. The mechanisms by which host immune system lose the supervision and clear the virus are poorly understood. When the HCV invades the body, under the stimulation of the viral proteins, the immune system is accurately activated and regulates the balance between inflammatory injury and immune tolerance. The standard treatment with pegylated interferon and ribavirin has limited effectiveness for the most prevalent viral genotypes (1a/1b) in the U.S in the past years, but the direct-acting anti-viral (DAA) treatment could enhance the effectiveness greatly [9]. While the unaffordable cost and the drug resistance also restrict its widespread use. Unlike HBV infection, no vaccine is currently available to prevent the HCV infection, which is a serious problem we must confront. To understand the HCV-host interactions that lead to viral persistence will help vaccine development and new drug design. The exact mechanism by which HBV escapes immunity is still not known. Interacting with virions or virus proteins, monocytes/macrophages play an important function in the disease process. The role of monocytes/macrophages in HBV and HCV infections is discussed in this chapter.

2. Monocytes/macrophages in HBV infection

Monocytes originated in the bone marrow mainly consist of a part of the innate immune system. Being the first immune barrier, monocytes play multiple roles in the immune system [10]. Such roles include: (1) act as a pool of precursor cell, which replenish to resident macrophages and dendritic cells as soon as needed, and (2) in response to signals of cytokines or chemokines, monocytes can be recruited and migrated to the sites of infected tissues quickly and divided/differentiated into local inflammatory macrophages and dendritic cells to trigger an immune response [11]. Whether HBV infection is cleared or persists is determined by host immune responses [7]. Viral replication takes place inside hepatocytes as soon as infection

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begins. The secretion of infectious virions or virus proteins can persist for decades at high rates. Hepatitis B virus (HBV) DNA, HBeAg and HBsAg can be easily detected in serum consequently. The levels of these clinical marker means HBV DNA, HBsAg for clinical diagnosis could fluctuate for a long time and keep sistency, are a reflection of virus duplicate activity and used to define the patients disease stage [9].

Circulating monocytes represent about 10% of mononuclear cells in human peripheral blood. Although involving in acute inflammation and wound, relatively little is known about the chronic viral infections on monocytes [12]. One research found that the function of monocytes were impaired in HIV and HCV infection, because of the responsiveness of TLRs [13–15], and Toll-like receptor responsiveness of monocytes in chronic HCV infections [14, 15]. Based on the surface of CD14 and CD16 expression, researches divided monocytes into two distinct subpopulations. $CD14^+$ $CD16^+$ monocytes, which occupy 10–20% of total blood monocytes and produces much more pro-inflammatory cytokines by the stimulation of TLRs ligands, such as TNF and IL-1β. The majority of CD14(high)$CD16^-$ (80–90%) monocytes have been reported to produce relatively high IL-10 and weak TNF [16, 17]. Hepatitis B surface Ag (HBsAg), as a main HBV protein, has been reported to suppress the activity of monocytes through binding to the monocytes. The binding to monocytes was enhanced by a heat-labile serum protein that was inhibited by Ca_2M/Mg_2M, low pH and an HBsAg-specific monoclonal antibody [18]. Hepatitis B surface Ag (HBsAg) inhibits monocytes inflammatory response by means of COX-2 dependence and may regulate natural killer (NK) cell function interfering IFN-γ production by inhibiting IL-18 and IL-12 production [19]. Hepatitis B surface Ag (HBsAg) is the most abundant HBV protein in the liver and in the peripheral blood of chronic hepatitis B (CHB) patients, which can accumulate up to 100 mg/mL in the peripheral blood, and typically outnumbers infectious virions by 1000:1 to 10,000:1 [20]. The high concentration of HBsAg in the blood stream of CHB patients could theoretically contribute to the hampered immune response. Several studies have shown that HBsAg can suppress the release of LPS-induced cytokines in human monocytes by interfering with the TLR signal pathway [21]. These results suggest that HBsAg could be consumed in the macrophages or Kupffer cells (KCs) (shown in **Figure 1**) and alter the innate immune response, which may contribute to the establishment of chronic infections.

Monocyte subset frequencies are altered depending on the clinical phase of the chronic HBV infection [23]. Moreover, HBeAg also plays a regulating action in the HBV infection.

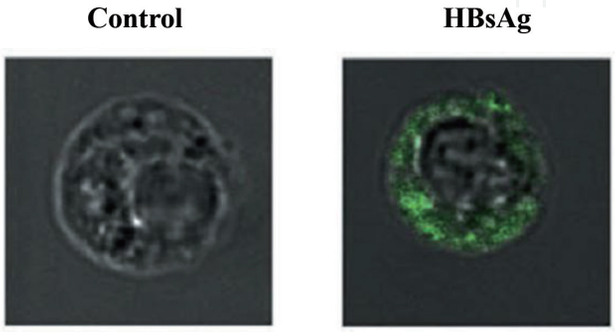

Figure 1. KCs uptake of HBsAg (green) *in vitro* imaged by confocal microscopy [22].

Expression of TLR2 correlates with HBeAg concentration negatively in CHB patients. Stimulation with TLR2 agonists *in vitro* peripheral blood mononuclear cell (PBMC) from HBeAg-positive patients produced less TNF and IL-6 compared to HBeAg-negative patients [24, 25]. Furthermore, exposure of monocytes to HBsAg suppressed LPS-induced TNF and IL-1β production *in vivo* and *in vitro* [26, 27]. Hepatitis B surface Ag (HBsAg) was related to decreased cytokine production induced by the TLR2 ligand (Pam3csk4) in PBMCs from chronic hepatitis B patients *in vivo* [28]. The later research demonstrates that HBsAg selectively inhibits Pam3csk4-stimulated IL-12 production. The mechanism study shows that HBsAg could inhibit JNK-MAPK pathway and provides a mechanism by which HBV evades immunity and maintains its persistence [21].

Kupffer cells (KCs) are the most important innate immune cells in the liver and constitute more than 80% of tissue resident macrophages in the body. Kupffer cells (KCs) account for about 15% of total liver cells, which are more than T cells and liver NK cells. Acting as scavenger cells, KCs remove particulate material from the portal circulation, which has been studied for a long time [29]. Viral infections have been implicated with the KCs in the pathogenesis of inflammatory liver diseases recently [30]. The KCs play an important role in liver injury when the liver is infected by HBV [31].

The liver is continuously exposed to non-pathogenic antigens (from food) and to gut derived lipopolysaccharide (LPS). The LPS is a powerful stimulus for innate immunity through TLR ligation and similarly activates professional antigen-presenting cells (APCs). Kupffer cells modulate the host immune response by the elaboration of IL-10. However, the pro-inflammatory cytokines (IL-12, IL-15 and IL-18) secreted by the Kupffer cells could stimulate NK cell function for secreting IFN-γ [32]. Kupffer cells (KCs) are intravascular macrophages that are continuously exposed to, and tolerant of, bacterial TLR ligands, which are delivered via the portal circulation.

In HBV infection, Kupffer cells participate in many immune responses, including immune cell activation, anti-viral immunity and tissue damage repair [33]. The immune cells cross talk occurs in the infected liver. Kupffer cells regulate T-cell responses by means of the co-stimulatory molecules CD80 and CD86, which are expressed on the cell surface [34]. The ligands of the two molecules on the T cells are CD28 and CTLA-4, respectively. CD86 on APCs stimulates T cells by binding CD28, which occurs before CD80 up expression. CD80 has a higher ability to initiate inhibitory signals through its interaction with CTLA-4 [35–37]. On the other hand, CD80 and CD86 regulate T helper cell differentiation and control adaptive immunity. CD80 mainly drives T-cell differentiation towards a Th1 profile and CD86 leads the differentiation towards a Th2 profile [38–42]. T-cell response in HBV infection mainly initiates Th2 immune response rather than Th1. The production of IL-10 was reported in many researches but the Th1 responses and cytokine production are weak when compared with resolver [43, 44].

Kupffer cells (KCs) and infiltrating monocytes/macrophages are main APCs to regulate adoptive immune response but must avoid hyper-activation of the immune system through expressing inhibitory molecules PD-L1 and PD-L2. The levels of PD-L1 and CD80/CD86 signals on APCs control the magnitude of T-cell activation [45–47]. One study has investigated

the expression of CD80 and CD86 on KCs in HBV infection. This study found that only few KCs express these molecules [48]. The different expressions of CD80, CD86 and PD-L1 in KCs in the portal areas of the liver were explored together with the correlation of their expressions with the fibrosis score and grade of inflammation during HBV infection. The HBV virus and the protein function on monocytes/macrophages are listed in **Table 1**.

Virus/protein	Cell receptor	Cell signal	Cytokines
HBsAg	TLR2	MAPK/JNK	IL-12
	TLR4	PI3K	TNF-a, IL-1b
HBeAg	TLR2	C-Jun/JNK	TNF-a, IL-6
HBVcore	TLR2/4	PI3K/JNK	TNF-a, IL-6,IL-12
HBV DNA	TLR7/9	RGI	IL-12, IL-18

Table 1. Monocytes'/macrophages' function in HBV infection.

3. Monocytes/macrophages in HCV infection

Hepatitis C virus (HCV) is also a cause of serious liver diseases as well as other extra hepatic pathologies especially in the developed countries. A classical combination of the drugs pegylated interferon and ribavirin used as standard treatment for decades. Recently, some other drugs have been approved that may enhance the effectiveness to a great extent. These drugs are designed to block the virus duplication by interfering with specific viral proteins. Rational therapies and effective vaccine can be designed if HCV replication is understood totally. Monocytes appeared to contain the core protein of HCV by flow cytometry *in vitro* infected experiment, suggesting that HCV was inside the monocytes [49]. Different types of monocytic cell lineages have been investigated, with $CD14^{+}$, $CD16^{++}$ and $CD14^{++}$, $CD16^{++}$ but not with $CD14^{+}$, $CD16^{-}$ cells being found infected [50]. These studies suggest that different cell types allow replication of slightly different versions of HCV.

Monocytes are not only infected by HCV virions, but also influenced by virus proteins. Chronic activation of monocytes and macrophages is seen in HCV and correlates with liver damage [51, 30]. Monocytes had the highest gal-9 levels in chronically infected HCV patients suggests that they may be a source of T cell inhibitory gal-9 in HCV infection. Toll-like receptors (TLR) have a critical role in innate immunity against pathogens. During chronic HCV infection, interleukin-12 (IL-12) produced by monocytes/macrophages is significantly suppressed. Programmed death-1 (PD-1), an inhibitory receptor on immune cells, plays a pivotal role in suppressing T-cell responses during chronic viral infection. IL-12 production decreased on monocytes/macrophages in HCV infection correlates the up-regulation of PD-1 on cell surface. IL-12 production resumed when PD-1/PD-L1 antibody or IFN/RBV treatment was carried out. The possible mechanism is that the STAT-1 phosphorylation is enhanced during the treatment [52]. Tim-3, acting as a negative regulator, inhibits monocytes'/macrophages' function in HCV infection in some researches [53].

Myeloid-derived suppressor cell (MDSC) is a kind of inhibitory cell that originated in the bone marrow and was first identified as natural suppressor cell in tumour-bearing mice in the mid-1960s [54]. Myeloid-derived suppressor cell (MDSC) originated in the bone marrow and later differentiated/divided into granulocytes, macrophages or mature dendritic cells [55, 56]. Myeloid-derived suppressor cell (MDSC) migrates or accumulates in the tumours, spleen, bone marrow and blood under different pathological conditions. Based on the different cell surface markers and cell origin, the MDSC can be divided into monocyte (Mo), granulocyte and endothelial-committed subsets. Myeloid-derived suppressor cell (MDSC) represents a cell type that suppresses the function of other immune cells and creates a suppressive environment. Studies revealed that MDSC numbers correlate with T-cell frequency inversely in the peripheral blood [57]. T-cell responses can be suppressed by MDSCs through numerous mechanisms [58–60]. In patients with HCV infection, T-cell function is impaired according to the clinical observation. Hepatitis C virus (HCV) core protein and polyI:C induce TNF-α (pro-inflammatory cytokine), IL-10 (immunomodulatory cytokine) and IFN-γ (anti-viral cytokine) secretions from monocytes. Then, monocytes are reprogrammed to acquire or lose the immunosuppressive (MDSC) phenotype through these cytokines. Hepatitis C virus–induced Mo-MDSC production was attributed to the PI3K pathway via induction of IL-10 and TNF-α secretion [61]. The HBV virus and the protein function on monocytes/macrophages are listed in **Table 2**.

Virus/protein	Cell receptor	Cell signal	Cytokines
HCVcore	TLR2	MYD88	IL-6, IL-10
HCV RNA	TLR7/8	CD81	IL-1b, IL-12
HCV dsRNA	TLR3	ISG15/56	IFNα/β
NS3	TLR2	RGI	IL-1b, IL-6, TNF-α

Table 2. Monocytes'/macrophages' function in HCV infection.

4. Monocytes/macrophages in HBV/HCV co-infection

Despite their different replication strategies and life cycles, HBV and HCV have similar modes of transmission through bodily fluid, and both have developed highly successful ways to establish chronic hepatitis [62]. Liver injury and disease progression are thought to be driven by the interaction between viruses and host immune responses in both infections [63–66]. Given their similar modes of transmission, HBV/HCV co-infection occurs frequently in endemic areas although its prevalence is exactly unknown [67]. Between 2 and 10% of anti–HCV-positive patients also test positive for HBsAg, while 5–20% of patients with chronic HBV infection test positive for anti-HCV antibodies [68]. It has been reported that HBV/HCV co-infection leads to more severe liver disease and a higher prevalence of liver cancer than non-infection [69], but an inverse relationship between the replication of each virus within some co-infected patients has been noted [70–74]. Hepatitis B virus-Hepatitis C virus co-infection

involves complex viral interaction; HBV and HCV can replicate in the same cells *in vitro* with no evidence of interference between them [75, 76]. Therefore, the viral interference observed in HBV/HCV co-infected patients *in vivo* is probably due to indirect mechanisms mediated by innate and/or adaptive host immune responses.

Hepatitis B virus-Hepatitis C virus co-infection is a blank field because of lack of robust cell and animal experimental model. Our research group attempts to do some basic experiments in HBV-HCV co-infection. Human primary peripheral blood monocytes were cultured for 2 days in increasing concentrations of infectious HCV, infectious HBV or both viruses together. As expected, the HCV, HBV and the two viruses together suppressed the expression of HLA-DR and TRAIL on monocytes, and increased the expression of PD-L1 and the secretion of IL-10, similar to the effects of recombinant HCV. In addition, we also uncovered previously unknown immune suppressive effects in both HCV and HBV, and the two viruses together strongly suppressed the expression of genes encoding stat1 and stat2, thereby disabling IFN signalling. These findings help to explain the well-known propensity of both HCV and HBV to induce T-cell immune suppression and clonal exhaustion, but they do not explain the anti-HCV effect of HBV infection *in vivo*. Other cell types, or cytokines, or both, may be playing a role in association with HBV mediated suppression of HCV.

To identify cytokines that were secreted in response to HBV, but not HCV, we performed experiments in which viruses were titrated either alone, or against a fixed concentration of the other virus, and observed that HBV induced IL-1β and IL-12 secretion, while HCV did not. Furthermore, the presence of HCV did not suppress the induction of either IL-1β or IL-12 by HBV. Since cytokine expression is regulated by a number of genes, we next measured the expression level of the associated genes. We observed novel emergent properties when the two viruses were combined. That is, Nfkb1 was not induced by HBV alone. It was induced by low concentrations of HCV, but then suppressed by higher concentrations. However, with HCV and HBV combined, the expression of this gene was sustained across a wide range of viral titers. Similarly, the Ifr1 gene was not affected by HBV alone but was modestly elevated by HCV, but drastically elevated with HCV and HBV combined. The secretion of TNF-α was strongly induced by HBV, but not by HCV, and modestly suppressed in the presence of both viruses together. Based on experiments in which human blood monocytes were exposed to intact HBV, intact HCV, or both viruses together, our results showed that both viruses exert strong immunosuppressive effects.

5. Perspectives

Our understanding of the role of monocytes/macrophages in HBV or HCV is far from completion. Nevertheless, the anti-viral roles of monocytes/macrophages will be appreciated by binding and/or uptake of virus leading to immune recognition and the production of pro-inflammatory or anti-inflammatory mediators resulting in (1) activation of neighbouring cells, such as NK cells and $CD8^+$ T cells, (2) blockage in viral replication in hepatocytes and (3) attraction, activation and interaction with other immune cells, including ILCs, pDCs and Tregs, which will further increase the anti-viral and inflammatory response. In the early

phases after infection, the immune activating roles of monocytes/macrophages are beneficial to help the body cause anti-virus response and clear virus and dead cell. But the situation will turn to the other side if the infection develops into chronic. The strong immune activity may also contribute to tissue damage and the development of fibrosis, cirrhosis and HCC. Furthermore, immune regulatory functions of monocytes/macrophages have been described, which may counteract the development of effective anti-viral immunity and support viral persistence and related disease pathogenesis.

Intrahepatic macrophages become an interesting and complex cellular target for treatment options and hot point in viral hepatitis with the growing appreciation of the roles of intrahepatic macrophages in both protective and harmful responses. With the development of flow and cell sorting technology, identifying phenotypical and/or functional characteristics discriminating KC from infiltrating macrophages will become easy day after day. Despite the existence of a HBV, vaccine is used widely but the already HBV infected population also be at high risk of developing to liver fibrosis and liver cancer. The standard treatment with pegylated interferon and ribavirin has limited effectiveness for the most prevalent viral genotypes (1a/1b) in the U.S in the past years, but the DAA treatment could enhance the effectiveness greatly. Also, the unaffordable price and the drug resistance also restrict its widespread use. Unlike HBV infection, no vaccine is currently available to prevent HCV, which is a serious problem we must confront. To understand the HCV-host interactions that lead to viral persistence will help vaccine development and new drug design. The exact mechanism of monocytes/macrophages in HBV and HCV infection will provide us new insight into and confidence to overcome the virus.

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