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MINIREVIEW

Macrophages in Hepatitis B and Hepatitis C Virus Infections[▽]

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major health burdens worldwide, with over 300 and 170 million people, respectively, infected. HBV, a DNA virus, and HCV, an RNA virus, are both hepatotropic, and both lead to hepatitis in many patients, with potentially fatal complications, including hepatocellular carcinoma. A high proportion of HCV- and HBV-infected patients develop chronic infections characterized by absent, weak, or narrowly focused T-cell responses (70). It is likely that early immune avoidance mechanisms contribute to the disturbed T-cell responses in combination with various other strategies reviewed elsewhere (7, 28, 31, 36, 70, 82). The two viruses differ considerably in their interactions with the host immune system, but all current treatment protocols aimed at clearing either virus include alpha interferon (IFN-α). This implies that the innate immune system is of pivotal importance in the development and maintenance of chronic infection versus viral clearance.

Critical components of the innate immune response are liver macrophages. Here we highlight their key roles in both the favorable and adverse responses to HBV and HCV infections.

MACROPHAGES IN THE LIVER

Macrophages are phagocytic mononuclear cells of the innate immune response which also prepare and maintain adaptive responses. Monocytes in peripheral blood differentiate into macrophages after migrating into tissues, where gene expression changes are driven by the extracellular matrix, chemokine milieu, and T cells (13, 47, 56). Kupffer cells (KCs), resident liver macrophages, are long lived and abundant, representing 15 to 20% of the total liver cell population (42, 68). Resting KCs, one of the first types of immune cells to be exposed to materials absorbed in the gut, contribute to the generally tolerogenic environment in the liver (3, 41, 68, 77, 86), including suppression of T-cell activation (51). Nevertheless, during immune responses, KCs, like circulating monocytes drawn into the liver, can be activated by various stimuli (17, 19, 22, 26, 34, 47, 57, 59, 64, 73, 77, 83) (Table 1). Low shear stress, fenestrations of sinusoidal cells, and a large contact area between blood and parenchymal cells facilitate extravasation and recruitment of immune cells to the liver (12, 43).

Macrophages display heterogeneous phenotypes with distinct functional capacities that vary according to tissue microenvironment and external stimuli. They monitor their environment and mediate phagocytosis via a plethora of plasma membrane receptors (90). Pattern recognition receptors, including Toll-like receptors (TLRs) (26, 90), recognize pathogen-associated molecular patterns and subsequently mediate both phagocytosis and signaling, leading to an altered macrophage phenotype (17, 22, 26, 34, 59, 64, 73, 83) (Fig. 1). Typically, innate immune activation is particle induced, antigen nonspecific, and T-cell independent (26, 47), resulting in macrophages that secrete reactive oxygen species (ROS), nitric oxide, type I IFNs (IFN-α and IFN-β), and other cytokines and chemokines. Additionally, innately activated macrophages often upregulate expression of adaptive response genes, such as the gene encoding antigen presentation major histocompatibility complex class II (MHC-II) molecules (42), and promote activation of primed T cells (26). In contrast, adaptive macrophage activation is modulated by direct interaction with T cells. Classical adaptive activation occurs in the presence of IFN-γ and results in macrophages with enhanced cytotoxic activity (19), whereas alternative adaptive activation, which is promoted by the type 2 cytokines interleukin-4 (IL-4) and IL-13, results in macrophages with activities optimized for combating parasitic and extracellular pathogens (26, 55). Other activation phenotypes, including that for IL-10-mediated activation, have been described (Table 1), but IL-10 has also been associated with deactivation of macrophages, which is necessary to limit the duration and intensity of the immune responses that downregulate MHC-II and the expression of other cytokines (26, 47).

MACROPHAGE INVOLVEMENT IN IMMUNE RECOGNITION OF HCV AND HBV INFECTIONS

In the case of HBV infection, viral replication inside infected hepatocytes occurs within capsids, with the viral genome hidden from pattern recognition receptors (PRRs), preventing the initial HBV infection from being detected by the innate immune system (95) (Fig. 2B). In contrast, the HCV life cycle is cytoplasmic in replication complexes. Although these membranous webs may shield the virus somewhat from PRRs, ubiquitously expressed cytoplasmic PRRs that detect nucleic acids, such as RIG-1 and MDA-5, likely recognize HCV and trigger secretion of detectable amounts of type I IFNs by hepatocytes (84, 85). Indeed, in studies with chimpanzees, no hepatic tran-

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scription changes are detected during the first weeks of HBV infection, whereas many hepatic transcription changes relating to the type I IFN response are seen upon HCV infection (70). There is debate as to whether macrophages, in addition to hepatocytes, are infected by HCV and support replication (4, 9, 20, 44, 69, 72, 74). If infected, macrophages may also contribute to the early induction of type I IFN signaling through RIG-1 and MDA-5.

HCV, unlike HBV, spreads rapidly in the liver. Therefore, it is likely that during the early stages of HCV infection and in the later stages of HBV infection, KCs (and other cells) are exposed to free viral nucleic acids and proteins. KCs and macrophages express TLR1 through TLR6 and TLR8 (87). Thus, TLR3 and/or TLR8 (located in late endosomes and lysosomes), which are activated by double-stranded RNA (dsRNA; TLR3) and single-stranded RNA (TLR8), likely recognize HCV via phagocytosis, become activated, and contribute to the induction of type I IFNs (Fig. 2). In the case of HBV infection, exactly which TLRs detect viral infection have not been delineated, though it is likely that phagocytosed viral particles detected by TLR8 trigger innate activation of macrophages. It has been suggested that HBcAg can bind macrophages via TLR2 and activate TNF-α, IL-6, and IL-12 expression (10), although in an immortalized human hepatocyte cell line transgenic for HBV it is specifically TLR3 agonists that inhibit HBV replication in isolated KCs through an IFN-β-dependent mechanism (97). The role of TLRs in combating HBV infection has been generally demonstrated by using a transgenic mouse model of HBV infection, where stimulation of most TLRs inhibits viral replication (33).

Several studies report that HCV can interfere with type I IFN signaling, in particular through interfering with pathways downstream of TLR3 (1, 30, 46, 62; reviewed in references 5 and 82) (Fig. 2A). Given that some type I IFN responses are observed in HCV infections, this finding suggests that the interference is not 100% but that the resultant response is too weak or too late to be effective. Nevertheless, therapeutic IFN-α can lead to the elimination of HCV in chronic infection.

Macrophages, including liver macrophages, upregulate their antigen-presenting phenotype in response to IFN-γ (88), leading to several-hundred-fold-increased expression of MHC-II (19). In HCV infection, most KCs express high levels of MHC-II molecules compatible with this phenotype (8, 38). MHC-II allows antigen presentation to CD4 T cells, but macrophages are also able to cross-present viral epitopes to CD8 cells. Here, cell-associated particulate viral antigen and viral dsRNA are taken up by macrophages, degraded in phagosomes, and cross-presented via MHC-I (2). This cross-priming or cross-presentation could provide an efficient means for viral antigens within dead cells to be presented to T cells by uninfected macrophages, thereby inducing adaptive immunity or facilitating activation of primed HCV-specific T cells (26). Indeed, cross-priming of viral dsRNA seems to be essential for the priming of cytotoxic T cells (76). However, in the absence of co-stimulatory molecules, cross-presentation of antigens by macrophages is more likely to promote tolerance in naïve T cells (3), which might play a role in HBV- and/or HCV-induced hepatitis, but data on these diseases are lacking.

In summary, HCV evades by antagonizing the immune re-

TABLE 1. Macrophages activation patterns in viral infection[a]

Activation	Stimulus(i)	Upregulated surface protein(s)	Downregulated surface protein	Intracellular response	Secretory response	Functional effects	References
Classical	TLR engagement and additional costimulation by IFN-γ may be needed for some responses	MHC-II, CD86		Upregulation of IRFs, iNOS, NO, and ROS	Production of inflammatory cytokines IFN-α, IFN-β, IL-1β, TNF-α, IL-12, and IL-6 and chemokines IL-8, CCL2 CCL3, and CXCL10	Enhanced AG presentation; type 1 effector response[b]	47,59
Alternative	Type 2 cytokines IL-4 and IL-13	Mannose receptor MHC-II	CD14	Downregulation of iNOS and NO	Upregulation of IL-1Ra, IL-10, and CCL18; downregulation of TNF-α, IL-6, IL-1β, and IL-12	Decreased AG presentation, decreased killing of intracellular organisms, increased AG endocytosis, T-cell proliferation, and increased extracellular matrix production[c]	17, 22, 26, 34, 59, 73, 83
IL-10	IL-10/IL-10 receptor	Mannose receptor PRRs (FMLP, TLRs, and MARCO); cytokine receptors (IL-12Rβ, IL-10R, IL-2Rα, and IL-21R)			Upregulation of IL-1Ra; downregulation of cytokine and chemokine production, MHC-II, CD80, CD86, and NO	Profound inhibition of AG-presenting and effector functions; remodeling of extracellular matrix	26, 38, 47, 57, 64

[a] Abbreviations: FMLP, formyl-methionyl-leucyl-phenylalanine; MARCO, macrophage receptor with collagenous structure; IRF, IFN regulatory factor; iNOS, inducible nitric oxide synthase; NO, nitric oxide; AG, antigen.

[b] Enhanced killing of intracellular pathogens and proinflammatory activation of adaptive immune response (e.g., induction of IFN-γ).

[c] Regulatory and recovery function and immunosuppression.

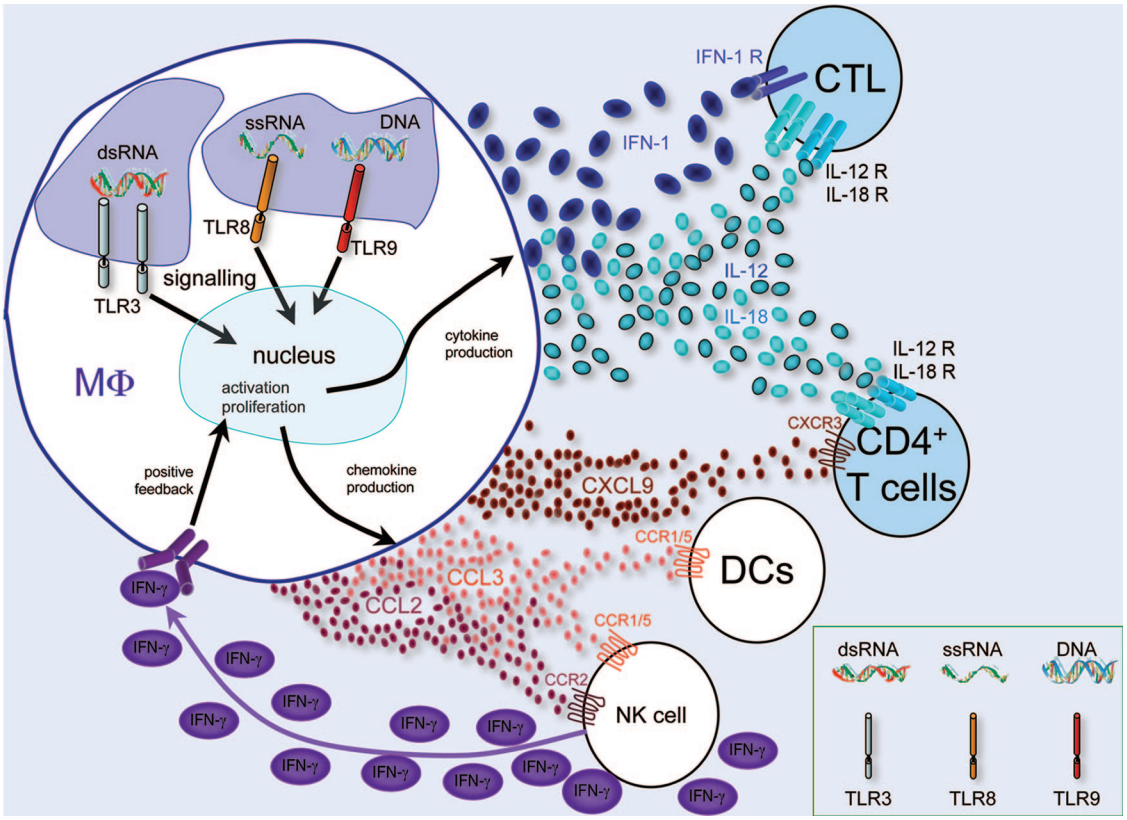

FIG. 1. Macrophages in viral infection. Phagocytosed viral DNA and/or RNA is recognized by the endosomal TLRs TLR3 (dsRNA), TLR8 (single-stranded RNA [ssRNA]), and TLR9 (DNA), which lead to signaling, transcriptional changes, and macrophage activation and proliferation, as well as to antigen presentation (not shown). As a result, cytokines (IFN-1, IL-12, and IL-18) and chemokines (CCL2, CCL3, and CXCL9) are produced, recruiting further immune cells of the innate and adaptive immune systems (NK cells, DCs, and T cells). Positive feedback via NK cell-produced IFN-γ (purple ovals) leads to further macrophage activation. The macrophage-produced cytokines type I IFN (IFN-α and IFN-β), IL-12, and IL-18 stimulate a type 1 immune response in $CD4^+$ and $CD8^+$ T cells. Recruited DCs present viral antigens, including activated $CD8^+$ cytotoxic T cells, to T cells, destroying infected cells (not shown). MΦ, macrophage.

sponse, whereas HBV hides from detection by the early immune system, including macrophages, and both viruses lead to persistent liver infections in many cases.

MACROPHAGES DETERMINE CELLULAR AND CYTOKINE MILIEU IN HCV- AND HBV-INFECTED LIVERS

In the murine cytomegalovirus (75) and *Listeria monocytogenes* (11, 42) models of liver disease, it has been shown that KCs and macrophages integrate a cascade of innate inflammatory events bridging the innate and adaptive immune responses: type I IFN-dependent CCL2 produced by KCs recruits circulating monocytes to the liver, where they produce CCL3 (75). CCL3 recruits NK cells producing high levels of IFN-γ, which triggers widespread classical activation of macrophages, inducing CXCL9 production, which in combination with CCL3 promotes recruitment of $CD4^+$ T cells (75, 99). Additionally, based on general immunology research, immune responses are expected to be further amplified by KCs and macrophages as follows: KC-secreted IL-12 and IL-18 should ensure that virus-specific $CD4^+$ T cells differentiate into type 1 helper (Th1) cells, which secrete IFN-γ, creating an activating feedback loop between KCs and Th1 cells and promoting infiltration of more T cells (47) (Fig. 1); type I IFNs, released from hepatocytes (and KCs), should promote the expansion and activity of cytotoxic $CD8^+$ T cells (84). Cell-to-cell interactions between macrophages and T cells which are complementary to the primary T-cell receptor-MHC-II interaction by CD40 (on macrophages) and CD40L (on T cells) should result in bilateral signaling; the macrophages produce more IL-2, TNF-α, and nitric oxide and increase MHC-II expression, while the Th cells produce more IFN-γ (47, 50).

Such a cascade would be difficult to demonstrate in human HCV or HBV infection. However, in HCV-infected chimpanzees, a comparable IFN-α response is found in all animals, but resultant chemokine- and IFN-γ-induced genes are correlated with viral elimination (85). This suggests that the early immune cascade that will include KCs is crucial in determining outcome. Not surprisingly, histological analysis of chronically HCV-infected livers demonstrates a dramatic increase in total as well as antigen-presenting macrophages in HCV infection (38, 53). Activated macrophages are found immunohistochemically in clusters with $CD4^+$ T cells (8). Furthermore, on microarrays, genes of macrophage activation are very prominent in chronic HCV-infected liver (80). KC-derived chemo-

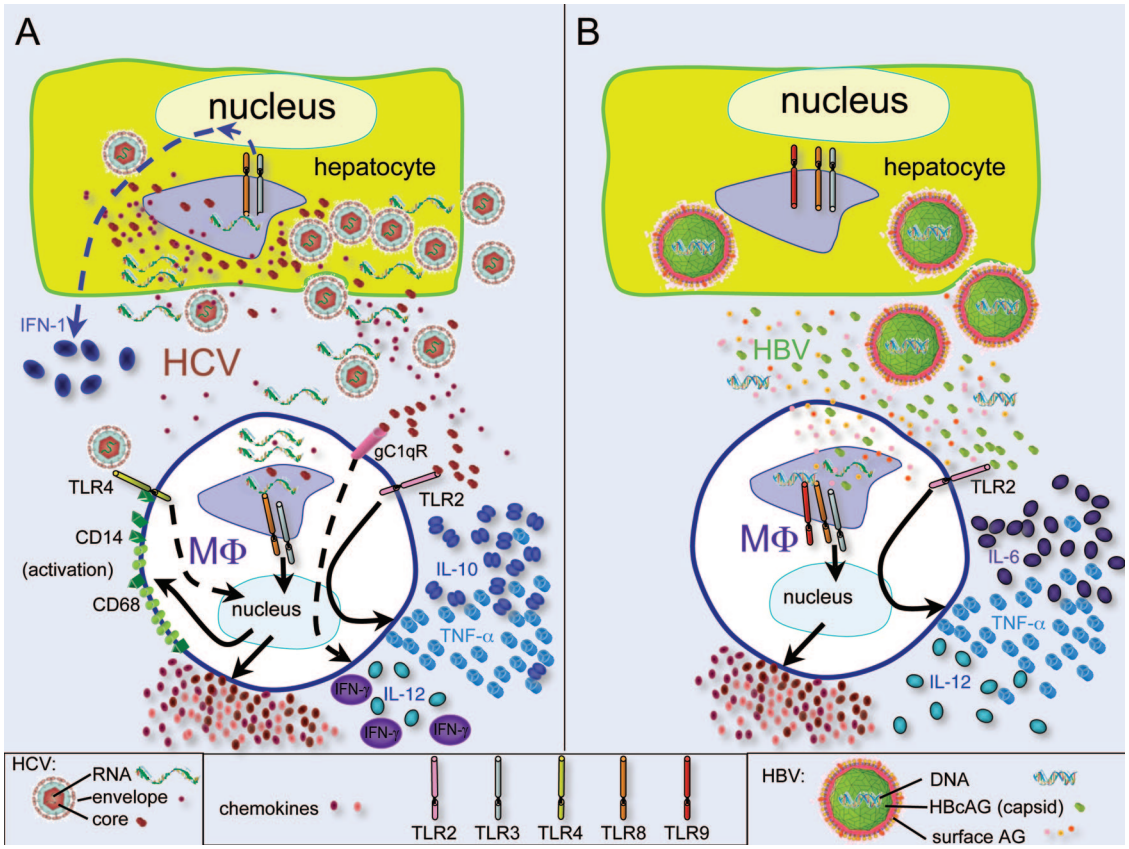

FIG. 2. Macrophages in HCV (A) and HBV (B) infections. (A) HCV is detected in hepatocytes by PRRs, including TLR3 and TLR8, and a cellular IFN response is triggered but blunted (dashed line) by viral interference at multiple points. Viral particles are released from hepatocytes and extracellular HCV RNA, and proteins influence macrophage biology either directly by binding (core/TLR2) or interference (gC1qR and TLR4) or through endosomal TLRs after uptake of viral particles. Macrophages become activated (increasing expression of CD14 and CD68) and produce chemokines and the cytokines IL-10 and TNF-α but less IL-12. These result in pDC apoptosis, decreased T-cell proliferation, and fibrogenesis (not shown). (B) In hepatocytes, HBV replicates within nucleocapsid particles, escaping detection by hepatocyte PRRs. Therefore, there are no transcription changes in the hepatocytes. Viral particles are released from hepatocytes and HBV DNA, and proteins influence macrophage biology through intracellular and surface receptors. Viral HBcAG (capsid) interferes with TLR2, leading to increased cytokine (IL-6, IL-12, and TNF-α) production. HBV particles taken up by macrophages are recognized by endosomal TLRs, leading to chemokine production, resulting in recruitment of antigen-nonspecific mononuclear cells, NK cells, and pDCs (not shown). MΦ, macrophage.

kines (CCL3) are important in the recruitment of dendritic cells (DCs) (52, 99) and NK cells (which correlate with the outcome of HCV infection [39]). Moreover, KCs regulate DC migration into Disse's space and to lymph, which is vital for antigen presentation and the development of adaptive immune responses (63, 99).

A number of studies indicate aberrant macrophage function in chronically HCV- and HBV-infected individuals. In HBeAg-positive HBV infection, TLR2 expression in KCs is reduced (93), perhaps suppressing immune surveillance. Also, activated macrophages produce less TNF-α in vitro when exposed to lipopolysaccharide (TLR4 ligand) in the presence of HBV particles (61), and specifically, HBsAg binds to macrophages, inhibiting lipopolysaccharide-induced macrophage activities such as IL-12 production. Similarly, via interactions with gC1qR (a surface complement receptor), the HCV core protein inhibits TLR4-induced production of IL-12 by macrophages (94). Peripheral blood monocytes taken from HCV-infected patients preferentially express IL-10 upon exposure to recombinant HCV, which may contribute to reduced T-cell responses (96) and exhibit defective responses to TLR3 and TLR4 ligands (92). It is currently not clear whether these cells are functionally impaired by the virus or whether they have adapted an altered and reversible phenotype due to external stimuli (plasticity). Evidence for a direct effect is HCV core protein binding surface-expressed TLR2, which has been suggested to bias macrophage activation toward secretion of IL-10 and TNF-α (14, 16) (Fig. 2A). This cytokine combination is proposed to predispose plasmacytoid DCs (pDCs) to apoptosis and low IFN-α production when exposed to TLR9 ligands (CpG DNA). The HCV core (32, 58), NS4A, NS4B (35), and NS5A (6, 25, 37, 66) have all been shown to induce CXCL8 expression in vitro. CXCL8 is elevated in the sera of HCV-infected patients (35, 54, 67, 71) and may inhibit the antiviral activity of IFNs (35, 66) and/or exacerbate inflammation (32, 35). Furthermore, when mixed with T cells, HCV core protein-expressing macrophage cell lines are less effective at promoting proliferation and IFN-γ production in T cells (45).

In summary, HBV and HCV, via an assortment of mechanisms, disturb immune responses and establish chronic infections, with macrophages as key regulators of the early immune responses being targeted by both viruses.

CONTRIBUTION OF MACROPHAGES TO LIVER INFLAMMATION

Characteristic pathological features of chronic HBV and HCV infections are chronic inflammation and apoptosis of infected and bystander hepatocytes (70, 82). KCs and macrophages are thought to be major contributors of bystander killing. In HCV infection, recently recruited ($MAC387^+$) macrophages are increased in the focal areas of erosions (40, 53), and KC-derived IL-18 correlates with hepatitis and liver injury (53). Similarly, in HBV infection, more activated KCs expressing FasL are observed during episodes of liver damage (89), and activated KCs together with oval cells have been described in areas of inflammation and regeneration (81). Indeed, CD68 (macrophage marker) and CD14 (activated macrophages [27]) are both upregulated in viral hepatitis and correlate with liver injury (49, 53, 91, 98). It has been proposed that in chronic HCV infection, a combination of viral (e.g., serum HCV core protein) and host (e.g., IFN-γ and unphagocytosed endotoxins) factors cause KCs and recruited macrophages to be continuously and inappropriately activated (15). Activated KCs potentially kill hepatocytes via several mechanisms. KC-derived TNF-α is known to be injurious to hepatocytes in various models of T-cell-dependent acute liver damage (65), and KC expression of FasL may also promote hepatocyte apoptosis (42, 65). Local production of nitric oxide and ROS by activated macrophages may also contribute to bystander hepatocyte cell death (42).

MACROPHAGES INFLUENCE LIVER FIBROSIS AND CIRRHOSIS AND CANCER

Important complications of HBV and HCV infections are fibrosis and cirrhosis and the development of hepatocellular carcinoma (HCC). KCs produce profibrogenic factors (e.g., transforming growth factor β and platelet-derived growth factor) (77) and yet also represent a major source of enzymes and factors important for matrix breakdown and turnover (collagenase, metalloproteinases, and IL-6) (64, 78, 79). Whether KCs exert a pro- or antifibrogenic effect depends on their cytokine environment (18, 21) and on interactions with stellate cells and hepatocytes (42). The serum- and glycocorticoid-regulated kinase is induced by transforming growth factor β and is associated with fibronectin formation, and in HBV and HCV infections, serum- and glycocorticoid-regulated kinase is upregulated in activated KCs, suggesting a profibrotic effect in these diseases (24). In a transgenic mouse model of HBV infection with hepatic HBV envelope protein expression, KCs exhibiting high levels of superoxides are observed specifically beside proliferating hepatocytes (29). HCV core- and NS5A-induced ROS and nitric oxide produced by activated KCs may also promote DNA damage in hepatocytes and induce oncogenesis (48). In addition, it has recently been postulated that KC-derived IL-6 contributes to hepatocyte proliferation and HCC development and that estrogen inhibition of IL-6 production reduces HCC risk in females (60). Therefore, KCs are not only relevant in HBV- and HCV-induced inflammation but also in the development of associated fibrosis and HCC. One study of KC depletion leading to attenuated liver injury (23) suggested an adverse net effect of liver macrophages, but the possible influence of macrophages will depend on their phenotypes and activation status.

CONCLUDING REMARKS

Liver macrophages and KCs contribute to the tolerogenic environment of the undiseased liver and influence every stage of virus-induced liver disease. They recognize viral antigens and initiate the innate immune response, of which they are a major component. KCs determine the cellular and cytokine milieu of the virally infected liver and thus play a major pathological role in chronic virus-associated diseases. Future research is necessary to answer many of the unresolved questions relating to macrophages in HCV and HBV infections, such as the following. Are monocytes and macrophages productively infected by HCV, and is this a significant parameter in immune escape by HCV? Do liver macrophages function efficiently during HCV and/or HBV disease, and can they be a target for immune therapy to control HBV and/or HCV disease?

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