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Review

The host–pathogen interaction during HBV infection: immunological controversies

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HBV is a hepatotropic and non-cytopathic virus that causes more than one million deaths annually from liver cirrhosis and hepatocellular carcinoma. As the virus itself is non-cytopathic, it is widely accepted that both viral control and liver pathology are mediated by the host immune system. Until recently, the focus has been on the crucial role of adaptive immune responses in controlling HBV infection, but the potential contribution of the innate system is now an important area of controversy. Unanswered questions include whether and when HBV can trigger components of innate immunity, and whether HBV can actively suppress the induction of innate immunity. We discuss the data available from animal models and human HBV infection addressing the role of innate immunity in the first part of this review. In the second part, we address the immunopathogenesis of the inflammatory events that characterize chronic hepatitis B. The mechanisms thought to be responsible for liver inflammation, namely the intrahepatic recruitment of inflammatory cells, which is orchestrated by chemokines, have been described; however, the underlying immunological triggers are much less clear. The prevailing idea is that liver inflammation results from a recovery of HBV-specific T-cells directly causing liver injury, but this scenario is supported by scanty experimental data. By contrast, recent findings raise the possibility of a contribution from innate components, such as natural killer cells.

Introduction

The host–virus relationship is often described by a series of organized virological and immunological events [1]: viral entry, propagation of the virus in the host and early activation of innate immunity followed by maturation of adaptive immunity that can control the infection and/or cause pathological damage of the infected organ. The tendency to apply this general scheme to distinct viruses is justifiable because viral propagation, followed by a generic 'immune activation', undoubtedly occurs with similar kinetics and magnitude in many viral infections; however, some viruses, such as HBV, have evolved ways to interact with the host that cannot be easily reconciled with this simplified view. The delayed amplification of HBV replication and spread after infection, the absence of early clinical symptoms of acute viral infection (fever and malaise) and the ability to persist in the infected host with the production of extremely high quantities of viral proteins (hepatitis B surface antigen [HBsAg] and hepatitis B e antigen [HBeAg]) are features peculiar to HBV [2,3]. The contrast with other viral infections is striking: most viruses (for example, HCV, HIV, human cytomegalovirus [HCMV], influenza and dengue) enter a logarithmic phase of propagation immediately after infection, resulting in febrile symptoms (for example, in HIV, HCMV, dengue and influenza) linked with high proinflammatory cytokine production [4]; if the virus persists, infection is typically associated with low viral load and protein expression (for example, in HCV, HCMV and Epstein–Barr virus).

In this review we critically discuss aspects of HBV immunopathogenesis, stressing divergences with standard dogma rather than trying to reconcile them with current views of host–virus interactions. The immunology of HBV has been comprehensively reviewed previously [3,5,6], as have potential novel immunotherapeutic approaches to the treatment of HBV [7,8]. Here, we focus our attention on two controversial issues in the HBV field: the role of innate

immunity in HBV infection and the significance of the term 'immune reactivation' in chronic hepatitis B.

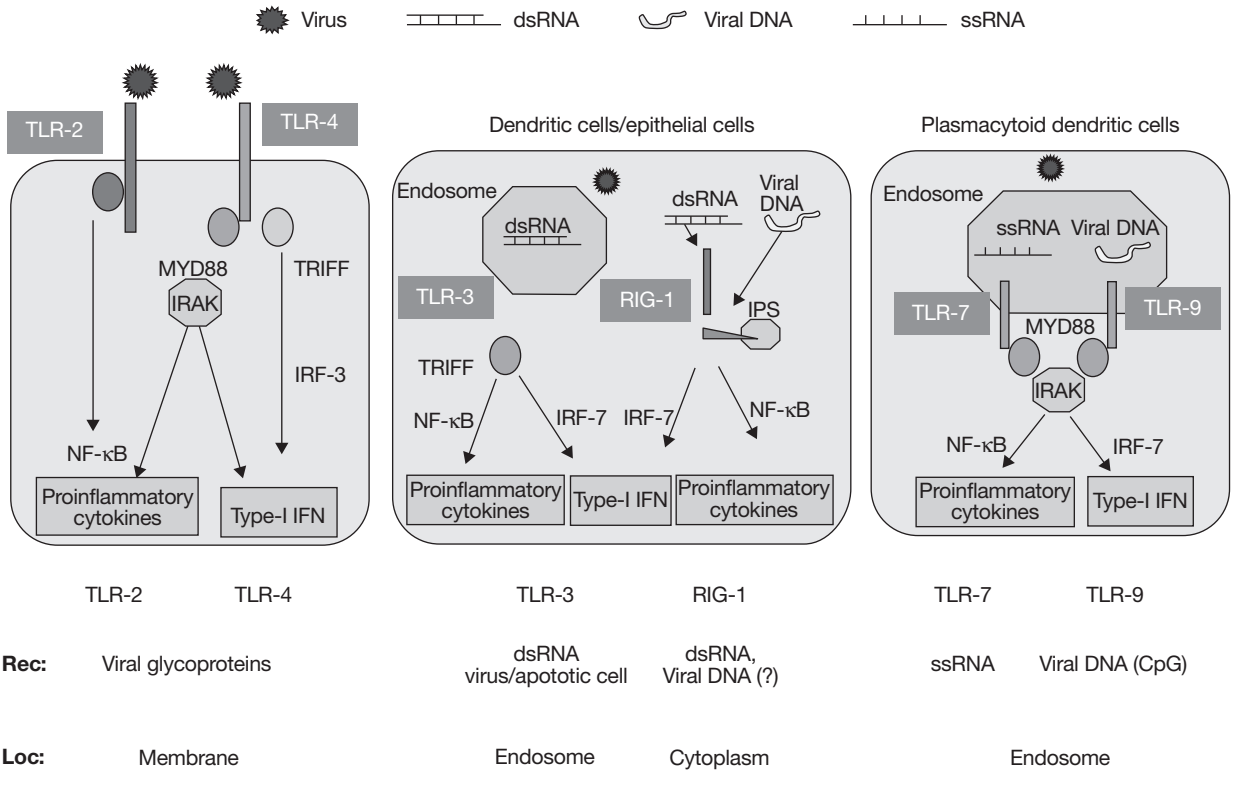
Controversy 1: the role of innate immunity in HBV infection

Innate immunity is made up of different components that sense the presence of invading pathogens. It elicits rapid and potent production of proinflammatory cytokines and chemokines that limit virus replication and induce maturation of adaptive immune responses. Mammalian cells mainly detect viral infections through the engagement of endosomal Toll-like receptor (TLR) -3, -7/8 and -9, or cytoplasmic receptors (Figure 1). These intracellular receptors recognize structural viral components like viral DNA, double-stranded RNA, single-stranded RNA and surface glycoproteins as pathogen-associated molecular patterns (PAMP). PAMP recognition by intracellular receptors triggers type-I interferon (IFN) production, which makes a key contribution to the initial inhibition of viral replication [9]. A major cellular component of innate immunity contributing to the initial containment of viral infection are natural killer (NK) cells, which are able to recognize and kill viral infected cells [10]. NK cell effector function is determined by the balance of signals from activating and inhibitory receptors. Loss of major histocompatibility complex class I on the surface of virally infected cells, along with up-regulation of host or pathogen-encoded ligands that signal cell stress, combine to increase the ratio of activatory over inhibitory signals, and optimize NK cell recognition. NK cells can also be directly activated by cytokines, such as IFN-I and interleukin (IL)-12, induced in viral infections [11].

Antiviral potential of innate immunity in HBV

The antiviral potential of these innate responses has been clearly demonstrated in animal models of HBV infection. NK cell activation, or activation of antiviral mechanisms by TLR ligands, efficiently inhibit HBV replication in transgenic mice [12–14] and cause a

Figure 1. Schematic representation of different cellular mechanisms of viral detection



The mechanism involved in HBV detection by infected hepatocytes or professional antigen presenting cells is unknown. dsRNA, double-stranded RNA; IFN, interferon; IPS, interferon promoter stimulator; IRAK, interleukin-1 receptor-associated kinase; IRF, interferon regulatory factor; Loc, localization; MYD88, myeloid differentiation primary response gene 88; NF, nuclear factor; Rec, recognition; RIG-1; retinoid-inducible gene-1; ssRNA, single-stranded RNA; TLR, toll-like receptor; TRIFF, Toll-receptor adapter inducer of interferon.

transient, but profound, suppression of hepatic viral load in woodchucks [15]. Similarly, activation of intracellular innate responses in human hepatocyte-derived cells (Huh7 and HepG2) through overexpression of pattern recognition receptor adaptor molecules (Toll and IL-1 receptor domain-containing adaptor, common myeloid differentiation factor 88 and IFN-β promoter stimulator 1) is extremely efficient in inhibiting HBV replication, although the antiviral effect cannot be mimicked by the simple addition of cytokines (IFN-α and IFN-γ) [16].

The actual involvement of these different components of innate immunity during natural HBV infection is, however, extremely controversial. Some studies show a profound inability of innate immunity to detect HBV infection [17], whereas others support an opposing scenario [15,18]. These conflicting results are likely to be a reflection of the major limitations encountered when trying to study the early immunological events during HBV infection. The data are, therefore, few and derived from very different experimental systems.

Limitations of existing model systems

Results obtained from patients after infection with HBV are limited by the difficulty in recruiting patients at the earliest pre-symptomatic stages of acute infection. Typically, studies of patients with acute HBV infection recruit donors who are already at the onset of acute symptomatic hepatitis, which occurs at least 6–8 weeks after innoculation. Most patients who have been studied at earlier time points still do not intercept the initial eclipse phase of HBV infection [19–21]. Similarly, an *in vitro* infection system for HBV in non-transformed hepatocytes is not widely available, its infection efficiency is around 20% of cultured cells and the level of HBV replication is low [22,23]. Animal models of HBV infection certainly provide good physiological data, but are hampered by ethical issues and high cost (for example, chimpanzees) or by a scarcity of reagents to analyse immunological events (for example, woodchucks) [15,24]. The practical consequences are that experimental reproducibility in chimpanzees is limited [17,25], whereas in woodchucks quantitative data of protein expression are often lacking, making it difficult to quantify the magnitude of events analysed only at the transcriptional level [15].

Type-I interferon responses in acute HBV

Bearing in mind these limitations, we will describe the conflicting data regarding innate immunity in HBV infection and then attempt to find some unifying interpretations. The hypothesis that HBV infection does not trigger a type-I IFN (IFN-I) response in the liver in the early phases of infection is derived mainly from data using experimentally infected chimpanzees, but has also been supported by recent analyses of proinflammatory cytokine production in humans during the early phases of infection [4,20] and during HBV reactivation after therapy withdrawal [26].

Three chimpanzees were infected with a single dose of a monoclonal inoculum of HBV (10^8 genome equivalents). All three animals developed a self-limited infection after virus spread to almost 100% of hepatocytes. Starting at week 1 after infection, the authors analysed viral and clearance-related gene expression, searching for up- or down-regulated genes that correlated with the amount of HBV DNA produced in the liver. Surprisingly, no altered expression of genes during the lag phase of HBV infection was found, suggesting that HBV does not directly activate innate immunity in the liver, but acts instead as a 'stealth virus' [17]. The data are particularly interesting because they contrast sharply with results obtained with a similar experimental approach performed in HCV-infected animals to detect activation of IFN-stimulated genes [27]. This highlights how two viruses able to infect the same organ and establish persistent infection utilize different strategies in their relationship with the host [2].

Supporting the data in chimpanzees, a recent report analysed the quantity of serum proinflammatory cytokines in 10 patients within the first 30 days of infection with HBV. Interestingly, a low proportion of tested patients experienced elevations of proinflammatory cytokines, and when elevations were detected they were of lower magnitude and delayed kinetics compared with HIV- and HCV-infected patients [4]. A lack of induction of IFN-I was also seen in a cohort of 21 acute HBV patients, 8 of whom were sampled repeatedly from the inception of the viral expansion phase through to viral contraction and resolution of liver inflammation [20].

The described inability of HBV to trigger a robust innate immune response is in agreement with the paucity of 'flu-like' symptoms experienced by patients with acute HBV infection and/or HBV reactivation, and by the lack of viral interference in HBV–HCV coinfection experiments [28]. These findings could be attributable to the replication strategy of HBV. The HBV transcriptional template (covalently closed circular DNA) is sequestered within the nucleus of infected cells, it produces polyadenylated viral messenger RNA (mRNA) that resembles the normal cellular transcripts and the viral replicative genome is protected within viral capsids in the cytoplasm [2]. Thus, a lack of IFN-I induction might reflect the capacity of HBV to escape the usual cellular sensing machinery.

Alternative innate antiviral cytokines

The apparent lack of induction of IFN-I raises a number of questions. For example, what is instead responsible

for the early control of HBV in the 'eclipse phase' and how does HBV escape this to replicate to such high levels? Is it possible that the prototypic early antiviral role of IFN-I is partially replaced by type-III IFNs that are preferentially produced in certain tissues including the liver [29] and have been demonstrated to have anti-HBV activity in the HBV transgenic mouse [30]? No significant induction of IFN-λ (IL-29) was observed in the viral ramp-up phase of acute HBV in humans [20], but data on the activity of this and other members of the IFN-λ family of cytokines are not available from donors immediately after inoculation. In light of recent studies showing a strong association between single-nucleotide polymorphisms in the IFN-λ non-coding region with HCV clearance [31–33], and previous data linking coding changes in a gene encoding a receptor for IFN-λ with HBV clearance [34], the potential role of these IFNs in early control of HBV merits further study. Another cytokine recently shown to have the capacity to inhibit HBV replication is IL-6, which is induced by non-parenchymal cells in response to HBsAg produced by primary human hepatocytes replicating HBV [35]; however this induction is only transitory, with the non-parenchymal cells rapidly becoming refractory to further stimulation [35], in line with data suggesting that HBV proteins can inhibit TLR-mediated signals [36].

Innate and adaptive interactions

Overall, available human data indicate a striking lack of induction of IFN-I [20] and proinflammatory innate cytokines in the early phase of HBV compared with HIV and HCV [4]. This is interesting given the finding that most adults control acute HBV infection successfully and suggests IFN-I might not be essential either for direct antiviral effects or for successful maturation of adaptive immunity in this infection. A 'danger signal' is a prerequisite for the maturation of adaptive immunity [37] and IFN-α production has been suggested to be indispensable for intrahepatic antigen-specific $CD8^+$ T-cell activation [38]. However, IFN-I has also been reported to impair adaptive responses, acting through signal transducer and activator of transcription 2 to inhibit dendritic cell development and expansion [39]. In addition, an intense, early cytokine storm has been viewed as detrimental in HIV infection, because of its ability to promote excessive immune activation with immunopathological consequences [4]. This concept is supported by new data from primates infected with simian immunodeficiency virus, showing that animals that rapidly contain the IFN-I response manifest a non-pathogenic infection, whereas those developing pathogenic infections exhibit sustained innate activation driving T-cell exhaustion [40,41]. In these models, the findings pointed towards an active regulation of innate immune response rather than an intrinsic attenuation. A number of regulatory mechanisms could be implicated, including those mediated by adaptive immune responses themselves [42], such that a weak innate response might be tempered by a strong, early adaptive response rather than *vice versa*.

Lack of induction and/or suppression of innate immunity

This leads on to another question arising from the lack of *in vivo* detection of IFN-I in acute HBV. Is there a possibility that this is also partially attributable to active suppression, rather than a complete failure of induction? Data indicating that HBV does not completely lack the inherent capacity to induce IFN-I come from the study of HepaRG cells infected with a recombinant baculovirus carrying the HBV genome. This expression system achieves high intracellular HBV replication levels and potently induces IFN-I-stimulated genes, resulting in non-cytolytic suppression of HBV replication [18]. Thus, it is conceivable that high-level HBV replication in isolated hepatocytes can induce IFN-I, but that other non-parenchymal cells in the hepatic milieu have the capacity to switch this off, so that IFN-I is not significantly induced *in vivo* [43]. Similarly, Kupffer cell production of IL-6 in response to HBV-infected primary human hepatocytes was rapidly down-regulated [35], implicating a viral evasion strategy to inhibit this antiviral cytokine [36].

Non-parenchymal liver cells do not support active HBV replication [44] and it is unclear how they sense the presence of HBV. Activation of murine non-parenchymal cells with TLR agonists can suppress HBV replication [45]. Some viral envelope proteins or other viral components can be recognized by pattern recognition receptors expressed at the cell surface (TLR-2 and -4). However, detection of viral products by TLR-2 and TLR-4 mostly results in the production of proinflammatory cytokines (IL-6, IL-8 and tumour necrosis factor [TNF]-α), but not IFN-I [9]. HBV secretory viral proteins (HBsAg and HBeAg) can abrogate the TLR-induced innate response [35,36] and modulate the surface expression of TLR-2 [46]. The possibility that secretory proteins of HBV (HBsAg and HBeAg) can interact and modulate the inflammatory environment of the liver directly or through the induction of immunosuppressive cytokines, such as IL-10, is gaining some experimental evidence [20,46]. Patients with HBeAg-negative chronic hepatitis B (CHB) manifest more aggressive liver disease, a clinical observation that could reflect the ability of HBeAg to down-modulate the proinflammatory liver environment. The production of HBeAg and HBsAg has been associated with interference of adaptive immune mechanisms [47], but a careful evaluation of their effect on innate immunity is still needed.

Natural killer cell responses in acute HBV infection

What role do NK cells play in acute HBV infection? Even without an appropriate induction of activatory cytokines, NK cells remain well poised to respond to acute HBV infection in the liver. Hepatocytes express very low levels of major histocompatibility complex class I such that there would be minimal engagement of inhibitory NK cell receptors, and any upregulation of cellular stress ligands able to engage NK activatory receptors should be able to induce local NK cell effector function. Furthermore, NK cells are extremely abundant in the liver, constituting 30–40% of intrahepatic lymphocytes [48,49]. In chimpanzees, clearance of HBV-infected hepatocytes by adaptive immunity was preceded by an increase in intrahepatic IFN-γ and TNF-α [50], which could have been produced by NK cells; however, subsequent experiments pointed to a crucial role for T-cells rather than NK cells in HBV control in this model [51]. Studies of patients around the time of first detection of HBsAg and HBV replication revealed an increase in the number of circulating NK cells [19,21], but their activation and effector function was suppressed as viral load increased and only peaked once viraemia had resolved [20]. This inhibition of NK cell activation and effector potential showed an inverse temporal correlation with an induction of IL-10, again raising the possibility that HBV can actively evade immune responses [20]. Induction of IL-10 was not detected in patients with an asymptomatic mild course of acute HBV [20], in line with the lack of attenuation of NK activity at peak viraemia in patients with this clinical pattern of disease [21]. No data are available from humans immediately after HBV inoculation regarding the involvement of NK cells in the immediate response to infection. However, recent results obtained in woodchucks infected with high doses of woodchuck hepatitis virus (10^{11} copies/ml) show induction of a gene related to NK cell activation immediately after infection (8–12 h) [15]; therefore, it remains possible that NK cells contribute to HBV control in the earliest lag phase of infection. NK cells might also contribute to liver damage in acute HBV infection, as there is an increase in the $CD56^{bright}$ subset that can induce hepatocyte death through the TNF-related apoptosis-inducing ligand (TRAIL) [48] coinciding with peak liver inflammation [20].

Reconciling conflicting data

The importance of size and speed of viral replication are starting to be appreciated as important parameters in the virus–host relationship [25,52] and it is interesting to see that, in HBV infection models, innate mechanisms are activated only by robust and brisk increases in levels of HBV replication. HepaRG cells need robust HBV expression to activate IFN-stimulated genes [18], and woodchucks showing early NK cell gene induction were infected by a very high single dose of virus [15]. Perhaps the slow pace of HBV amplification in the infected liver, characterized by a long (3–4 week) lag phase, is insufficient to activate innate immunity, which might be triggered not by the recognition of HBV molecular patterns, but perhaps by cellular stress signals induced by high intracellular production of HBV antigens. This interpretation could potentially reconcile the opposing views present in the literature, although it depicts a scenario where innate immunity is not acting as an early recognition system, but as a type of inflammatory trigger activated by high levels of HBV expression. This speculation is supported by clinical observations. For example, hepatic flares of CHB are usually preceded by high levels of HBV reactivation, which trigger production of proinflammatory cytokines (that is, IFN-α, IL-8 and chemokine ligand [CXC motif ligand; CXCL-10]) with [48] or without [26] detectable NK activation, suggesting that intrahepatic proinflammatory cytokines can be triggered by HBV viral antigen accumulation. Such speculations will have to be properly analysed and a definitive answer about the ability of innate immune responses to detect HBV replication will not be achieved without the definition of the specific cellular receptors responsible for innate recognition of HBV.

It seems clear that a better understanding of the ability of the innate immune response to control HBV infection during natural infection awaits data generated in more physiological systems. It is, for example, important to remember that analysis of the anti-HBV efficacy of IFN-α in HepG2 or Huh-7 hepatocyte-derived lines might be altered by the presence of defects in the IFN signalling pathways of such transformed cells. Currently, the majority of data seem to indicate that during natural HBV infection, characterized by low infectious dose and natural HBV amplification, innate immune activation of an intracellular antiviral response is rather weak. Interestingly, this scenario is present not only in acutely infected chimpanzees [17] and humans [4,20], but also during HBV reactivation after therapy withdrawal [26]. As a possible consequence of this low ability to be detected by intracellular innate mechanisms, HBV does not seem to have developed robust mechanisms to inhibit the antiviral effects of IFN-I. The efficacy of NK cell activation in the control of HBV is also difficult to quantify. The presence of activated NK cells in the early stages of acute infection might limit initial HBV spread, but their antiviral efficiency is unknown in the natural setting. Finally, the role of innate immunity must not only be restricted to its direct antiviral mechanisms. Production of proinflammatory cytokines and NK cell activation has an important effect on the correct triggering and

efficiency of adaptive immune responses, which in turn might feedback on innate responses. These functional aspects of innate immunity have so far received little attention in HBV infection. However, mediators with clear immunomodulatory effects on T-cell function and inflammatory cell recruitment (such as IL-10, IL-8 and serotonin) are becoming recognized as factors that play an important role in the immunopathogenesis of HBV infection [20,48,53]. Clearly, a large quantity of work awaits a new generation of brave scientists ready to accept the challenge of disentangling the complex interaction between HBV and the immune system.

Controversy 2: immune reactivation in CHB

The natural history of CHB is conventionally divided into distinct phases, supposedly related to their 'immunological activity' [54]. 'Immunotolerant', 'immune clearance', 'inactive carrier state' and 'reactivation' are terms that define CHB patients with particular clinical and virological profiles, but their actual adherence to a defined immunological profile is highly questionable. For example, the term 'immunotolerant phase' defines the period mainly present in children and young adults characterized by the presence of HBeAg, high serum HBV DNA and normal alanine aminotransferase (ALT). There is no doubt that adult patients with this virological and clinical profile have a highly defective HBV-specific T-cell response; however, a similar immunological profile is also found in chronic HBV patients with high HBV DNA and increased levels of ALT who are arbitrarily classified in the 'immune clearance' group [55,56]. Furthermore, the term HBV immunotolerance in children is often used to support the idea that children vertically infected with HBV cannot mount an effective immune response against HBV. In reality, the profile of antiviral immune response in HBV-infected children is unknown and we would argue that the efficacy of HBV vaccination in children born from HBV-infected mothers [57,58] is a direct demonstration that vertically infected patients can mount an efficient HBV-specific immune response and thus should not strictly be defined as immunotolerant. Similarly, inactive HBV carriers are indeed the patients from whom HBV-specific T-cell responses are most likely to be detected, both from the liver and circulating compartments [59]. The term inactive seems appropriate for the clinical, but certainly not for the immunological profile.

The role of T-cells in HBV flares

Immune-based terminology is also problematic in the use of the term 'immune reactivation' in CHB. This phase of the complex natural history of HBV disease coincides temporally with an increase in pathological activity in the liver (manifested as an increase in the level of ALT). The prevailing idea is that 'immune reactivation' is the result of a quantitative recovery of the HBV-specific T-cell response [60]. The scientific rationale for this scenario is valid because HBV is considered a fully non-cytopathic virus and adoptive transfer of HBV-specific T-cells in HBV-transgenic mice is able to trigger liver damage [61]; however, the data derived from patients experiencing such acute flares are few and somewhat contradictory.

An increment in $CD4^+$ T-cell proliferation against the whole hepatitis B core antigen was detected around the time of hepatic flares in some studies [62,63], but these findings could not be confirmed in recent analyses where different assays (intracellular cytokine staining or human leukocyte antigen [HLA] tetramer quantification) were used to quantify HBV-specific $CD4^+$ and $CD8^+$ T-cell responses [26,55,59]. It is important to note that in a recent analysis of a small cohort of patients developing hepatic flares after therapy withdrawal, an increase in HBV-specific T-cell responses was only detected in one patient who controlled HBV infection and did not develop a hepatic flare [26].

Two other important points need to be taken into account. Firstly, the frequency of circulating and intrahepatic HBV-specific $CD8^+$ T-cells in patients with chronic HBV infection correlate with HBV control and not with liver damage [59]. Secondly, work in animal models has clearly shown that the bulk of liver damage is not directly caused by HBV-specific $CD8^+$ T-cells, but is mainly caused by their ability to trigger a series of secondary events including hepatic granulocyte infiltration, chemokine production and subsequent recruitment of an inflammatory infiltrate composed of macrophages, NK cells and T-cells [64,65]. As a consequence, there is no direct correlation between HBV-specific cytotoxic lymphocyte (CTL) frequency and the extent of liver damage, and hepatic flares cannot be simply associated with an increment of the HBV-specific T-cell response.

Amplification of liver damage by innate mechanisms

It is instead very likely that more complex processes are taking place during the phase of immune reactivation of HBV; two reports have recently demonstrated an increase in IL-8 levels at the time of the increase in viraemia preceding hepatic flares [26,48] and an increase in IFN-α coinciding with the onset of the flare itself [48]. IL-8 (CXCL-8) is a granulocyte chemotactic factor, and granulocytes are indispensable for the initiation of liver inflammation in HBV-transgenic mice [66]. IL-8 can also potently chemoattract NK cells [67] and up-regulate TRAIL death-inducing receptors on hepatocytes, whilst a subsequent increase in IFN-α can induce TRAIL expression on NK cells. This could allow NK cells from patients undergoing flares to kill hepatocytes through the TRAIL pathway [48]. Furthermore, increased levels of

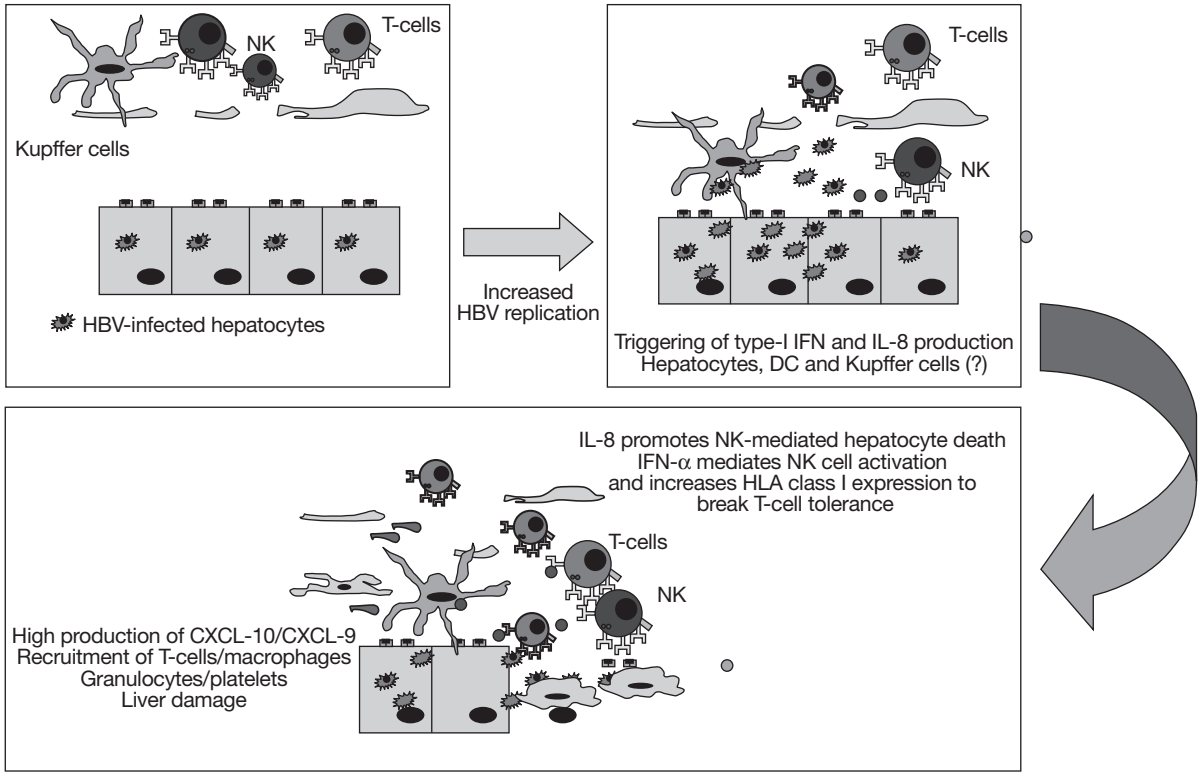
CXCL-10, CXCL-9 and other cytokines (such as IL-12) can be detected at the time of hepatic flares [68,69], as well as an increase in regulatory mechanisms, such as regulatory T-cells [70].

The temporal correlations of these different immunological parameters with the presence of hepatic flares does not, however, clarify what triggers them; most spontaneous flares are preceded by an increase in HBV DNA, but the cause of this increase remains elusive. The subsequent interactions of the different immunological mediators also remain difficult to unravel.

A plausible hypothesis (Figure 2) is that a sudden rise in HBV antigen expression could stimulate intrahepatic innate mechanisms able to break the state of immunological ignorance of the HBV-specific T-cells present in the liver. Several studies have shown that a sudden increase of HBV DNA and HBV antigens precedes hepatic flares [48,71–73]. We have discussed how activation of innate immunity might be linked to a sudden and excessive production of HBV antigens. In addition, data in transgenic mice have demonstrated the ability of intrahepatic IFN-α to break the immunological tolerance of intrahepatic antigen-specific T-cells [38]. It is thus possible to hypothesize that an initial activation of innate immune mechanisms (mediated by proinflammatory cytokines and/or NK cells) within the liver could alter HBV antigen presentation by hepatocytes and allow CTL recognition of HBV-infected hepatocytes. Hepatocytes express constitutively very low levels of HLA-class I [74] and are insufficient in antigen processing and presentation pathways, which can be augmented by intrahepatic IFN-α or IFN-γ production [75]. CTL activation will trigger CXCL-9 and CXCL-10 production, which are necessary for the accumulation of inflammatory cells in the liver and are a consistent finding in hepatitis reactivation in HBV-infected, HIV–HBV-coinfected and HCV-infected patients [26,69,76].

We should, however, keep in mind that, despite the plausibility of this 'reductionist' scenario, other components are likely to play a role. Activation of innate mechanisms might also modify intrahepatic CTL function independently of the quantity of HBV antigen presented by hepatocytes. Changes in the liver microenvironment, such as platelet activation, can increase CTL-mediated liver damage [53] and platelet activation can be triggered by IFN-I [77]. Conversely, intrahepatic

Figure 2. Pathogenesis of hepatic flares: a working hypothesis



CXCL, CXC motif ligand; DC, dendritic cells; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; NK, natural killer.

CD8+ T-cell function can be modulated by expression of programmed death ligand-1 molecules on infected hepatocytes [78–80], which have been shown to be increased by IFN-α or IFN-γ production [81]. Intrahepatic CD8+ T-cell function might also be modulated by the nutrient microenvironment; increases in arginase activity accompanying hepatic flares could deprive local T-cells of arginine and thereby bias their effector function towards a proinflammatory phenotype [82].

A conceivable alternative scenario is that the markedly depleted HBV-specific CD8+ T-cell response in patients with chronic HBV infection [55,56,59,83] allows an increase in viral replication triggering spontaneous hepatic flares; this then drives activation of innate mechanisms that mediate the flare itself. It is, in summary, extremely difficult to predict the complex web of interactions between immune cells, hepatocyte environment and HBV replication that take place in the liver during episodes of hepatic reactivation.

Clinical implications

A better understanding of the role played by the different components of the immune system in the different phases of HBV disease is necessary to design new treatments to eradicate this infection. Hopefully, understanding that hepatic flares of CHB are not necessarily and invariably caused by an increment of HBV-specific T-cell responses will start to remove the stigma associated with their role in HBV infection and facilitate the development of treatments aimed at boosting their antiviral capacity in this disease. It will be important to remember that the protective and pathogenic aspects of HBV-specific T-cells can be dissociated. This is illustrated by the high frequency of HBV-specific CD8+ T-cells in the livers of healthy carriers [59] and by the reduction of liver disease with viral control maintenance obtained by blocking the non-specific inflammatory infiltrate in HBV transgenic mice [64–66]. Ensuring that therapies aimed at boosting T-cells are carried out in the presence of potent antiviral suppression should prevent the initiation of the immunological sequelae leading to hepatic flares. Conversely, an understanding that a lack of increase in transaminases does not necessarily signify an absence of immune response against the virus, and that innate immune mechanisms might be instrumental in activating them, could open new avenues for therapies focused on the restoration of adaptive immunity.

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Disclosure statement

The authors declare no competing interests.

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