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Pathogenesis of Hepatitis B Virus Infection

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Abstract

The adaptive immune response is thought to be responsible for viral clearance and disease pathogenesis during hepatitis B virus infection. It is generally acknowledged that the humoral antibody response contributes to the clearance of circulating virus particles and the prevention of viral spread within the host while the cellular immune response eliminates infected cells. The T cell response to the hepatitis B virus (HBV) is vigorous, polyclonal and multispecific in acutely infected patients who successfully clear the virus and relatively weak and narrowly focussed in chronically infected patients, suggesting that clearance of HBV is T cell dependent.

The pathogenetic and antiviral potential of the cytotoxic T lymphocyte (CTL) response to HBV has been proven by the induction of a severe necroinflammatory liver disease following the adoptive transfer of HBsAg specific CTL into HBV transgenic mice. Remarkably, the CTLs also purge HBV replicative intermediates from the liver by secreting type 1 inflammatory cytokines thereby limiting virus spread to uninfected cells and reducing the degree of immunopathology required to terminate the infection.

Persistent HBV infection is characterized by a weak adaptive immune response, thought to be due to inefficient CD4⁺ T cell priming early in the infection and subsequent development of a quantitatively and qualitatively ineffective CD8⁺ T cell response. Other factors that could contribute to viral persistence are immunological tolerance, mutational epitope inactivation, T cell receptor antagonism, incomplete down-regulation of viral replication and infection of immunologically privileged tissues. However, these pathways become apparent only in the setting of an ineffective immune response which is, therefore, the fundamental underlying cause. Persistent infection is characterized by chronic liver cell injury, regeneration, inflammation, widespread DNA damage, and insertional deregulation of cellular growth control genes which, collectively, lead to cirrhosis of the liver and hepatocellular carcinoma.

Keywords

cytotoxic T cells; liver cirrhosis; hepatocellular carcinoma; innate immune response; adaptive immune response; chronic infection

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Introduction

Viral hepatitis is a necroinflammatory liver disease of variable severity. Persistent infection by HBV is often associated with chronic liver disease that can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). Many studies suggest that HBV is not directly cytopathic for the infected hepatocyte [1–3]. For example, during the early phase of HBV infection in chimpanzees (i.e., before virus-specific T cells enter the liver), 100% of the hepatocytes may be infected without histological or biochemical evidence of liver disease [4,5]. Furthermore, when cellular immune responses are deficient or pharmacologically suppressed, HBV can replicate at high levels in the liver of patients [and in immunologically tolerant HBV transgenic mice [3]] in the absence of cytological abnormalities or inflammation [6–8].

Viral clearance and disease pathogenesis are largely mediated by the adaptive immune response in HBV infection [2]. For HBV to persist it must either not induce a response or it must overwhelm, evade or counteract it. Interestingly, HBV “evades” the innate immune response by simply not inducing it, acting as a stealth virus in this regard [9]. On the other hand, viral persistence is characterized by a state of relative hyporesponsiveness of HBV-specific T cells [6,10–13]. Several viral proteins have been shown to regulate the adaptive immune response to HBV (as described below) suggesting that HBV may employ active evasion strategies targeting the adaptive immune response [1,5,6,10,13–19]. Indeed, it has been shown that antiviral treatment can overcome CD8⁺ T cell hyporesponsiveness in chronic HBV infection, suggesting that the T cells are present in these subjects but suppressed [20]. Importantly, a recent study suggests induction of an effective HBV specific CD8⁺ T cell response is dependent on early CD4⁺ T cell priming which might be regulated by the size of the viral inoculum [21].

Characteristics of the Immune Response to HBV

Absence of an Innate Response by Infected Cells

Virus replication often results in the induction of an innate immune response which is heralded by rapid induction of IFN α/β by the infected cell [22]. Production of IFN α/β induces the transcriptional expression of a large number of interferon inducible genes (ISGs) which in turn exert a variety of intracellular antiviral mechanisms that have the potential to minimize pathogenetic processes by limiting viral production and spread [22,23]. Surprisingly, as shown in Figure 1a, intrahepatic gene expression profiling in acutely HBV infected chimpanzees revealed that HBV acts like a stealth virus early after infection since it does not induce any cellular gene expression including ISGs as it spreads through the liver [9,23]. This is in stark contrast to the induction of many ISGs during the spread of hepatitis C virus (HCV) infection in chimpanzees, as shown in Figure 1b, suggesting that, unlike HBV, HCV is highly visible to the innate immune system [23–25]. The relative invisibility of HBV to the innate sensing machinery of the cells likely reflects its replication strategy, which retains the transcriptional template in the nucleus, involves the production of capped and polyadenylated viral mRNAs that resemble the structure of normal cellular transcripts, and sequesters its replicating genome within viral capsid particles in the cytoplasm [8,26,27]. Thus, the typical widespread expansion of HBV in the liver may reflect the absence of IFN α/β production to which the virus is exquisitely sensitive as has been shown in HBV transgenic mice [28,29].

The Adoptive Immune Response

The Antibody Response—The antibody response to the HBV envelope antigens is a T cell-dependent process [30]. Because these anti-envelope antibodies are readily detectable in

patients who clear the virus and recover from acute hepatitis, and they are usually undetectable in patients with chronic HBV infection, they are thought to play a critical role in viral clearance by complexing with free viral particles and removing them from circulation or by preventing their attachment and uptake by hepatocytes. This notion is supported by the observation that chimpanzees that resolved a previous infection are completely protected from rechallenge [31]. The appearance of neutralizing antibodies, however, occurs relatively late after HBV exposure and, thus, it is unlikely to contribute to the early phase of viral clearance during acute infection. Instead they probably prevent viral spread from rare cells that remain infected after resolution of HBV infection.

The CD4 T Cell Response—The peripheral blood CD4 T cell response to HBV is vigorous, and multispecific in patients with acute hepatitis who ultimately clear the virus, while it is relatively weak in persistently infected patients with chronic hepatitis [32]. Although the association between a strong CD4 T cell response, acute hepatitis, and viral clearance suggests that a relationship exists between these events [30,32,33], CD4 T cell depletion at the peak of HBV infection had no effect on viral clearance and liver disease in infected chimpanzees [5], suggesting that CD4 T cells do not directly participate in viral clearance and tissue damage. As we will discuss in more detail later in this review CD4 T cells probably contribute indirectly to the control of HBV infection by facilitating the induction and maintenance of the virus-specific (B cell) and CD8 T cell response.

The CD8 T Cell Response—The HBV specific CD8 T cell response plays a fundamental role in viral clearance and the pathogenesis of liver disease. A vigorous polyclonal CD8 T cell response is readily detectable in the peripheral blood of patients with acute hepatitis who ultimately clear HBV. In contrast, the peripheral blood T cell response in chronically infected patients is weak and narrowly focused [11,13,15,30]. The livers of these patients contain virus-specific T cells that likely contribute to disease pathogenesis, but for functional and/or quantitative reasons are unable to clear the infection. Interestingly, a recent study that examined a relationship between the number of intrahepatic HBV specific CD8 T cells, extent of liver disease, and levels of HBV replication in chronically infected patients indicated that inhibition of virus replication could be independent of liver damage, and that the functionality of HBV-specific CD8 T cells was more important than the number of T cells to control HBV replication [34]. Experiments in chimpanzees have shown that the viral clearance and the onset of liver disease coincide with the accumulation of virus-specific CD8 T cells and the induction of IFN γ and IFN γ -inducible genes in the liver [4,5]. Importantly, depletion of CD8 T cells at the peak of viremia delays viral clearance and onset of viral hepatitis until the T cells return, proving that the viral clearance and liver disease are mediated by virus specific CD8 T cells [5].

Mechanisms of HBV Clearance

It is widely believed that the CTL response clears viral infections by killing infected cells. CTL killing is an inefficient process, however, requiring direct physical contact between the CTLs and the infected cells. Thus, it may not be possible for CTLs to kill all HBV infected cells if the CTLs are greatly outnumbered as occurs during HBV infections in which as many as 10^{11} hepatocytes can be infected [4,5,21]. Thus, although the liver disease in HBV infection is clearly due to the cytopathic activity of the CTL response, viral clearance may require more efficient CTL functions than killing. Important insights into the pathogenetic and noncytopathic antiviral functions of the CTL response have come from studies in HBV transgenic mice that develop an acute necroinflammatory liver disease after adoptive transfer of HBsAg specific CTL clones [35–37]. In that model (Figure 2), the CTLs rapidly enter the liver and recognize viral antigen which triggers two events: (a) apoptosis of the hepatocytes that are physically engaged by the CTLs, and (b) secretion of interferon gamma

(IFN γ) which noncytopathically inhibits HBV gene expression and replication in the rest of the hepatocytes [37,38] by preventing the assembly of HBV RNA-containing capsids in the cytoplasm [29,39] in a proteasome-[40] and kinase-dependent [41] process. During this remarkable process, the viral nucleocapsids disappear from the cytoplasm of the hepatocytes [29,37] and the viral RNAs are destabilized by a SSB/La-dependent mechanism in the nucleus [30,42–44], yet the hepatocytes remain perfectly healthy [30,38]. Antibody blocking and knockout experiments in the HBV transgenic mouse model further demonstrated that the cytopathic and antiviral functions of CTLs are completely independent of each other [37]. These results suggest that a strong intrahepatic CTL response to HBV can suppress viral gene expression and replication noncytopathically.

Interestingly, it has been shown that HBV replication is also suppressed by the antiviral effects of interferon alpha/beta [28,29,45]. Indeed, in the transgenic mouse model at least, HBV replication is inhibited by any stimulus that induces IFN γ - or IFN α/β in the liver, including CD4⁺ T cells [46], NK and NKT cells [47], and other hepatotropic viral [48] parasitic infections [49,50] and toll-like receptor activation [51]. This raises the possibility that HBV infection can be controlled by many arms of the immune response, and perhaps explains why HBV infection is almost always self-limited in immunologically normal adults.

To investigate whether these principles apply to the clearance of HBV infection, we extended these studies to HBV-infected chimpanzees [4,5]. Intrahepatic gene expression profiling in these animals revealed that the early phase of clearance of HBV (Figure 1c), and parenthetically also for HCV (Figure 1d) [25], was temporally associated with the appearance of CD3, CD8 and IFN γ mRNA and other T cell-derived and interferon gamma (IFN γ)-stimulated genes, some of which might contribute to the noncytopathic inhibition described above [5,9,52]. These transcriptional changes were reflected by the influx of virus-specific CD8⁺ T cells into the liver [5]. But, although HBV replicative intermediates [5,53] decreased as much as 50-fold from peak levels during this time, there was little or no attendant liver disease, despite the fact that virtually 100% of the hepatocytes were infected [4,5] suggesting that noncytopathic mechanisms were active during this early phase of viral clearance. Interestingly, the HBV transcriptional template (cccDNA) that is not expressed in HBV transgenic mice also decreased 8 fold during the same period of time, suggesting that cccDNA could, at least partially, be eliminated from hepatocytes by a non-cytolytic mechanism [53]. Furthermore, we also showed that monoclonal antibody mediated depletion of CD8⁺ T cells (but not CD4⁺ T cells) at the peak of infection delayed the onset of viral clearance and liver disease for several weeks until the antibody titers waned and virus-specific CD8⁺ T cells became detectable in the liver [5]. Thus, we conclude that the principle of CD8-dependent cytopathic and noncytopathic clearance of HBV (Figure 2), which was discovered in the HBV transgenic mouse model [37], is operative in the context of the full fledged viral infection [4,5,53].

Mechanisms of HBV Persistence

Neonatal tolerance to HBV is probably responsible for viral persistence following mother-infant transmission [6]. Neonatal tolerance to HBV might be induced by the HBV precore protein (HBeAg) that has the capacity to cross the placenta and induce neonatal tolerance in HBV transgenic mice [54]. On the other hand, the basis for the inadequate immune response that is characteristic of adult onset chronic HBV infections is not well understood, and may in fact be multifactorial and be influenced by the size of the viral inoculum as described below. Potential contributing factors to HBV persistence in adult HBV infection include mutational escape leading to inactivation of B-cell and T-cell epitopes (reviewed in [2,23]) and specific inhibition of the adaptive immune response by viral proteins. For example,

HBeAg has been shown to suppress the antibody and T cell response to HBcAg in *adult* T cell receptor transgenic mice [55]. Thus, HBeAg may suppress immune elimination of infected cells by HBcAg-specific T cells and, thereby, contribute to viral persistence in chronically infected adults consistent with the clinical observation that viral mutations that preclude the production of HBeAg are often associated with exacerbations of liver disease and, sometimes, even with viral clearance in chronically infected patients [8,56]. The hepatitis B surface antigen (HBsAg) might also suppress immune elimination of infected cells by functioning as a high dose tolerogen since extremely high serum HBsAg titers in the mg per ml range are often seen in chronically infected patients [57,58] and chronically infected patients display absent or subnormal levels of HBsAg-specific CD8⁺ T cells [58]. In addition, HBV X protein, a transcriptional transactivator that is required for initiation of infection [59,60], can inhibit cellular proteasome activity when it is overexpressed [61] and thereby might interfere with antigen processing and presentation.

The Size of the Viral Inoculum Contributes to the Outcome of HBV Infection

Recent studies in HBV infected chimpanzees using a wide dose range of a single monoclonal HBV inoculum demonstrated that also the size of the viral inoculum contributes to the outcome of HBV infection [21]. As shown in Figure 3a–c, animals inoculated with 10^{10} , 10^7 and 10^4 genome equivalents (GE) of HBV cleared the virus within 8–30 weeks after its first detection, in a virus dose-related fashion similar to what we have previously observed in several other animals that had been inoculated with 10^8 GE HBV [4,5]. In contrast, both of the animals that were inoculated with 10^1 GE became chronically infected (Figure 3d and e), one of which (like many chronically infected humans) ultimately cleared the virus in the context of an acute disease flare 42 weeks after first detection, while the other remained heavily infected for at least 55 weeks at which point the study was terminated. This suggests that a virus dose window exists between 10^4 and 10^1 GE within which the host-virus dynamics favor persistent infections, while higher doses favor viral clearance. Importantly, viral clearance (Figure 3a–c) was heralded by early CD4⁺ T cell priming either before or at the onset of detectable viral spread, and it coincided with a sharply synchronized influx of HBV-specific CD8⁺ T cells into the liver and a corresponding increase in intrahepatic CD8 mRNA, serum ALT activity and histological evidence of acute viral hepatitis. Interestingly, the first detectable peripheral CD4 T cell response occurred during or before the phase of detectable viral expansion in the animals that cleared the infection in this study [21]. In contrast, the CD4 response was delayed until after the onset of viral expansion in the animals that developed persistent infection (Figure 3d and e) at which point the virus had infected 100% of the hepatocytes [21] and there was an uncoordinated influx of HBV-specific CD8⁺ T cells into the liver and a correspondingly asynchronous increase in intrahepatic CD8 mRNA and serum ALT activity [21].

Early Priming of the CD4 T Cell Response is Required for Viral Clearance

These fascinating results suggest that an early CD4⁺ T cell response to HBV infection is required to induce the CD8⁺ T cell response that clears the infection. Indeed, inoculation of an animal that was immuno-depleted of CD4⁺ cells with a virus dose (10^4 GE HBV) that should have been terminated in the context of a T cell response resulted in persistent HBV infection [21] (Figure 4). Importantly, CD4 T cell immunodepletion using the same antibody had no impact on the outcome of infection when it was delayed until 6 weeks after inoculation, i.e. at the peak of HBV infection, with the same inoculum in another chimpanzee [5]. Collectively, these results suggest that the timing of CD4⁺ T cell priming relative to the kinetics of viral spread is a key element in determining the magnitude and quality of the subsequent CD8⁺ T cell response to HBV and, therefore, the outcome of HBV infection. Since early CD4⁺ T cell priming in high dose HBV infections was observed in

some animals prior to detectable viremia (Figure 3b and c) and antigenemia [21], it was likely triggered by noninfectious subviral antigens that are in large molar excess (4 logs) relative to the number of infectious virions in the inoculum. Collectively, these results are consistent with the hypothesis that the early CD4⁺ T cell priming is required to activate professional antigen presenting cells necessary for the induction of efficient antiviral CD8⁺ cytotoxic T lymphocyte (CTL) responses [62,63]. In contrast, in the absence of early CD4⁺ T cell responses, CD8⁺ T cell priming could occur in the liver, which has been shown to result in T cell inactivation, tolerance or apoptosis [64–66].

Mechanisms of Hepatocarcinogenesis During HBV Infection

Multifactorial mechanisms contribute to the development of hepatocellular carcinoma (HCC) in chronic HBV infection. Both viral and host factors including genetic alterations induced by viral DNA integration, expression of oncogenic viral proteins and chronic immune-mediated hepatitis (Figure 5) have been implicated as contributing factors [1,2,67].

Integration of hepatitis B virus DNA

It has been long recognized that most tumors in HBV associated HCC contain clonally integrated HBV DNA and microdeletions in the flanking cellular DNA which could deregulate cellular growth control mechanisms [68]. Indeed, in HCCs developed in woodchucks infected with woodchuck hepatitis B virus (WHV), WHV DNA insertions predominantly occur in the N-myc2 oncogene which leads to N-myc2 activation [69–71]. Recent studies of HBV insertions in HBV-related HCCs revealed that also HBV integration can occur in genes that target telomerase and mixed lineage leukemia encoding genes suggesting potentially common pathways in HBV-related carcinogenesis [72–76]. HBV DNA integration has also been observed in patients with chronic hepatitis suggesting that integration is likely an early step in the process of hepatocarcinogenesis [77]. However, HBV integration is not likely occur in resting hepatocytes. Thus, if integration of the HBV genome contributes to hepatocarcinogenesis, it is likely to be secondary to procarcinogenic events that trigger hepatocyte turnover (e.g., immune-mediated hepatitis) as described below.

Role of viral proteins

In addition to integration, it is possible that certain HBV proteins may directly participate in HCC development. For example, the HBV X (HBx) gene product has been shown to transactivate cellular genes associated with cellular growth control [78–80]. The HBV X protein has also been shown to interact and interfere with numerous transcription factors [including Ca(2+)/cAMP-response element binding protein (CREB), activating transcription factor 3 (ATF)], nuclear factor interleukin- 6 (NFIL6), early growth response-1 (Egr1), Ets-1, octamer-binding protein (Oct1), and retinoid x receptor (RXR)], tumor suppressor genes (including p53), and proteins involved in DNA repair functions (including p53 and UVDDDB1) (reviewed in [67,81]). Furthermore, HBx sequences with C-terminal deletions and point mutations have been detected in chronic hepatitis and HCC [82,83], and since these mutations seem to arise before the development of HCC [84], these results suggest that deregulated X gene expression from integrated fragments of subviral DNA could play a role in hepatocarcinogenesis [67,85]. Similarly, a C-terminally truncated version of the HBV middle envelope protein (M-HBs) has been shown to be expressed from integrated subviral DNA in HCC and exert transcriptional activator activity [86,87]. Likewise, the HBV large envelope (L-HBs) has been shown to be a transcriptional transactivator [88]. Thus, these viral products, expressed from integrated viral DNA or replicating virus may contribute to hepatocarcinogenesis by their capacity to activate a variety of cellular promoters (including AP-1 and NF- κ B) [88–90].

Immune mediated liver injury

Almost all cases of HCC take place after many years of chronic immune-mediated hepatitis [6] characterized by continuous cycles of low-level liver cell destruction and regeneration that (over long periods of time) lead to fibrosis, cirrhosis, steatosis, and probably HCC. Indeed, transgenic mice that produce hepatotoxic quantities of L-HBs [91–94] display hepatocellular injury, regenerative hyperplasia, chronic inflammation, Kupffer cell hyperplasia, oxygen radical production, glutathione depletion, oxidative DNA damage, transcriptional deregulation and aneuploidy that inexorably progresses to HCC. [92,94–98] More importantly, transgenic mice that express nontoxic concentrations of the HBV envelope proteins in the hepatocyte and don't develop evidence of liver disease, develop HCC after many months of chronic hepatitis mediated by HBV-specific CTLs [99]. Importantly, the appearance of HCC in these settings occurs despite the absence of cofactors such as viral integration and X gene expression that have been discussed above. Since the immunological, virological and histological features of this chronic immune-mediated HCC model closely resemble the features of human chronic hepatitis, the results suggest that an ineffective immune response is a critical oncogenic factor during chronic HBV infection in man. The pathogenetic importance of immune-mediated hepatocellular injury in hepatocarcinogenesis in HBV is strengthened by the fact that hepatocellular carcinoma occurs in the context of necrosis, inflammation and regeneration (cirrhosis) in several human liver diseases other than hepatitis B, including chronic hepatitis C [100], alcoholism, [101] hemochromatosis, [102] glycogen storage disease, [103] α -1-antitrypsin deficiency, [104,105] and primary biliary cirrhosis. [106] Irrespective of etiology or pathogenesis, therefore, it would appear that chronic liver cell injury is a premalignant condition that initiates a cascade of events characterized by increased rates of cellular DNA synthesis and production of endogenous mutagens coupled with compromised cellular detoxification and repair functions. If these processes are sustained for a sufficiently long period of time, they would be expected to cause the multiple genetic and chromosomal changes necessary to trigger the development of hepatocellular carcinoma (Figure 5).

Summary and Conclusions

In summary, HBV acts like a stealth virus early in infection, remaining undetected and spreading until the onset of the adaptive immune response several weeks later. The relative invisibility of HBV to the innate sensing machinery of the cells probably reflects its replication strategy with the replicating viral genome being sheltered within viral capsid particles in the cytoplasm. On the other hand, HBV can be controlled when properly activated HBV-specific CD8⁺ T cells enter the liver, recognize antigen, kill infected cells, and secrete IFN γ which triggers a broad-based cascade that amplifies the inflammatory process and has noncytopathic antiviral activity against HBV. However, establishment of an effective adaptive antiviral immune response is dependent on CD4⁺ T cells and their priming early in infection most likely triggered by the subviral antigens present in the inoculum rather than by the infectious virions. Failure to trigger early CD4⁺ T cell responses, as occurs in low dose infections, induces functionally impaired CD8⁺ T cell responses resulting in the establishment of persistent infection. The inefficient immune response to HBV during chronic HBV infection results in low-level liver cell destruction and regeneration over long periods of time that lead to fibrosis, cirrhosis, steatosis, and eventually HCC.

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Figure 1.

Liver gene expression profile during HBV and HCV infection [9]. (A) Genes correlated with viremia in all acutely HBV infected chimpanzees. No genes correlated positively or negatively with the level of intrahepatic HBV DNA during the time course of acute HBV infection in all three animals. (B) Intrahepatic gene expression correlated with viremia in three HCV infected chimpanzees [107]. (C) Liver gene expression profile associated with viral clearance in all three acutely HBV-infected chimpanzees and (D) that associated with clearance in HCV infected chimpanzees [107]. Gene identities in HBV and HCV infected animals are described in Wieland et al [9] and Su et al [107], respectively. Blue and green lines show the intrahepatic HBV DNA or serum HCV RNA as a percentage (% max) of the corresponding peak levels, respectively. For optimal visualization, gene expression levels (red lines) are shown after normalization to the 10th and 90th percentiles for each gene. Values on the x axis represent weeks after inoculation with HBV. Adapted from Wieland et al. (2004) PNAS vol. 101 pp. 6669–74 and Su et al. (2002) PNAS vol. 99 pp. 11181–6.

Figure 2.

Noncytopathic clearance of HBV from the hepatocyte by T cell- derived cytokines. On antigen recognition, CD8-positive CTL deliver an apoptotic signal to their target cells, killing them. They also secrete IFN γ and TNF- α , cytokines that abolish viral replication and HBV gene expression in vivo, potentially curing them. Reprinted/amended from Am J Pathol 2000, 156:1117–1132 with permission from the American Society for Investigative Pathology.

Figure 3.

Peripheral CD4⁺ T cell responses against HBV core protein and intrahepatic CD8⁺ T cell responses in chimpanzees infected with a dose range of HBV as described in Asabe et al. [21]. The upper panel in each figure represents the serum HBV DNA as a black line and sALT as a yellow shaded area and the results of peripheral CD4⁺ T cell ELISPOT assays are overlaid as black bars. Cryopreserved PBMCs were thawed and stimulated in vitro with HBV core protein and the numbers of IFN γ producing cells were determined by ELISPOT assay as described [21]. The data are shown as number of spots at each time point minus the number of spots before inoculation per million PBMCs. The lower panel shows the total number of intrahepatic HBV-specific CD8⁺ T cells per 10² total CD8⁺ T cells as filled blue bars (right axis) and fold induction of intrahepatic CD8 mRNA compared to two preinoculation time points as a shaded red area (left axis). Intrahepatic lymphocytes were expanded antigen-nonspecifically in vitro and tested with all the corresponding Patr/peptide multimer complexes as described [21]. nt: not tested. *: tested and negative. Reproduced/ amended with permission from American Society for Microbiology from Journal of Virology, 2009, vol 83, pp. 9652–62, DOI: 10.1128/JVI.00867-09.

Figure 4.

Course of HBV infections, peripheral CD4⁺ T cell responses against HBV core protein, and intrahepatic CD8⁺ T cell responses in chimpanzees with or without CD4 immunodepletion as described in Asabe et al [21]. Serum HBV DNA levels are shown as a black line and sALT as a yellow shaded area. Horizontal bars represent serum HBe and HBs antigen levels and the open horizontal bars represent the presence of anti-HBc, anti-HBe, and anti-HBs antibodies. The amount of each protein is reflected by the thickness of each bar as indicated in the legend. The numbers of CD4⁺ T cells per μ L of whole blood were shown as closed squares (top panel, right axis). Arrows on the top panels represent injections of control antibody (a) or anti-CD4 antibody (b). Peripheral CD4⁺ T cell IFN γ ELISPOT assays against HBV core protein (second panel) and detection of intrahepatic HBV-specific CD8⁺ T cells (bottom panel, left axis) were as described in Figure 3 except that freshly prepared cells were used instead of using cryopreserved cells. Fold induction of intrahepatic CD8 mRNA compared to two preinoculation time points is shown as a shaded red area (bottom panel, right axis). *: tested and negative. Reproduced/amended with permission from American Society for Microbiology from Journal of Virology, 2009, vol 83, pp. 9652–62, DOI: 10.1128/JVI.00867-09.

Figure 5.

The chronic injury → HCC hypothesis. According to this hypothesis, a vigorous (+++) immune response to HBV leads to viral clearance while an absent (–) immune response leads to the “healthy” carrier state, and an intermediate (+) immune response produces chronic hepatitis. This indolent necroinflammatory liver disease is characterized by chronic liver cell necrosis which stimulates a sustained regenerative response. The inflammatory component includes activated macrophages that are a rich source of free radicals. The collaboration of these mitogenic and mutagenic stimuli has the potential to cause cellular and viral DNA damage, chromosomal abnormalities, genetic mutations, etc, that deregulate cellular growth control in a multistep process that eventually leads to hepatocellular carcinoma. Reprinted from *Am J Pathol* 2000, 156:1117–1132 with permission from the American Society for Investigative Pathology.