

REVIEW

The immune response during hepatitis B virus infection

Antonio Bertolotti and Adam J. Gehring

The UCL Institute of Hepatology, University College of London, 69–75 Chenies Mews, London WC1E 6HX, UK

Correspondence

Antonio Bertolotti
a.bertolotti@ucl.ac.uk

***Hepatitis B virus* (HBV) is a major cause of chronic liver inflammation worldwide. Recent knowledge of the virological and immunological events secondary to HBV infection has increased our understanding of the mechanisms involved in viral clearance and persistence. In this review, how the early virological and immunological events might influence the development of a coordinate activation of adaptive immunity necessary to control HBV infection is analysed. The mechanism(s) by which high levels of viral antigens, liver immunological features, regulatory cells and dendritic cell defects might maintain the HBV-specific immunological collapse, typical of chronic hepatitis B patients, is also examined.**

Introduction

Hepatitis B virus (HBV), a member of the family *Hepadnaviridae*, is a hepatotropic non-cytopathic DNA virus that despite the presence of an effective prophylactic vaccine is estimated to infect 300 million people, with a particularly high prevalence in Asia and Africa (Lok & McMahon, 2001).

HBV causes liver diseases that vary greatly in severity from person to person (Ganem & Prince, 2004). Some subjects control infection efficiently and clear the virus from the bloodstream either without clinically evident liver disease or with an acute inflammation of the liver (acute hepatitis) that can resolve without long-term clinical sequelae. Other patients fail to clear the virus and develop chronic infection. Most chronically infected patients remain largely asymptomatic without life-threatening liver disease but 10–30 % develop liver cirrhosis with possible progression to liver cancer (Alberti *et al.*, 1999; Lok & McMahon, 2001). The rate of HBV chronicity is low in adult infections (5 % or lower) but age and route of infection influence the outcome with exposure in neonatal life leading to a high rate of HBV persistence (Lok & McMahon, 2001; Ganem & Prince, 2004). Outcome of infection and the pathogenesis of liver disease are determined by virus and host factors, which have been difficult to fully elucidate because the host range of HBV is limited to man and chimpanzees.

The study of animal models of related hepadnavirus infections and transgenic mouse models able to express individual HBV genes or replicate the entire viral genome have clarified several aspects connected to HBV infection. Furthermore, the ability to analyse many immunological phenomena *ex vivo* through direct quantification of Ag-specific T cells in humans and chimpanzees has considerably increased our knowledge of HBV pathogenesis.

Here, we will not review the virological features of HBV, which have recently been covered in excellent reviews (Seeger & Mason, 2000; Wieland & Chisari, 2005), but discuss the pattern of HBV immunity and analyse how some virological features can influence it. We will then focus our attention on the distinctions of HBV immunity between resolved and persistently infected patients and the host/viral factors that can cause and maintain them.

Early events

Innate immunity generally plays a role immediately after infection to limit the spread of the pathogen and initiate efficient development of an adaptive immune response. Innate host responses during the early phases of viral infections are mainly characterized by the production of type 1 interferon (IFN)- α/β cytokines and the activation of natural killer (NK) cells. Production of type 1 IFNs can be triggered directly by virus replication through cellular mechanisms that detect the presence of viral RNA or DNA (Alexopoulou *et al.*, 2001; Lund *et al.*, 2003; Heil *et al.*, 2004), while NK-cells are activated by the recognition of stress-induced molecules and/or the modulation of the quantity of major histocompatibility complex (MHC)-class I molecules on the surface of infected cells (Moretta *et al.*, 2005).

The general pattern of fast viral spread and subsequent rapid activation of innate immunity has been deduced primarily from mouse models of different viral infections [*Lymphocytic choriomeningitis virus* (LCMV) and murine cytomegalovirus] (Biron, 2001; Ou *et al.*, 2001) and holds true for many human viruses like *Human immunodeficiency virus*, cytomegalovirus and Epstein–Barr virus. However, the simple observation of clinical, virological and immunological phenomena that follow HBV infection depicts a completely different and unconventional pattern (Fig. 1).

Experimental data collected, mainly in animal models but also in humans (Fong *et al.*, 1994), show that after inoculation, HBV does not immediately start to replicate efficiently. HBV-DNA and HBV antigens are not detectable in serum or the liver until 4–7 weeks post-infection (Berquist *et al.*, 1975; Korba *et al.*, 1989; Fong *et al.*, 1994; Guidotti *et al.*, 1999; Thimme *et al.*, 2003). Following this period, HBV begins a logarithmic expansion phase that can be detected in the liver and serum, reaches levels of 10^9 – 10^{10} copies ml^{-1} (Whalley *et al.*, 2001) and infects most hepatocytes (Jilbert *et al.*, 1992; Kajino *et al.*, 1994; Guidotti *et al.*, 1999; Thimme *et al.*, 2003).

The peculiarity of the kinetics of HBV replication has been largely ignored and only recently the comparison with hepatitis C virus (HCV) viral kinetics has drawn attention to the unusual pattern of HBV replication (Bertoletti & Ferrari, 2003; Wieland & Chisari, 2005). Rigorous experiments in chimpanzees showed that while HCV replication in the liver starts immediately after infection (Thimme *et al.*, 2002), larger doses of HBV inoculums do not enter an exponential phase of replication until 4–5 weeks after infection (Thimme *et al.*, 2003). The initial lag phase of HBV replication does not appear to be a consequence of HBV inhibition by elements of innate and adaptive immunity. The activation of IFN- γ , interleukin (IL)-2 and tumour necrosis factor (TNF)- α and intrahepatic recruitment of inflammatory cells is delayed until the logarithmic expansion of HBV in experimentally infected woodchucks (Cote *et al.*, 2000; Hodgson & Michalak, 2001; Nakamura *et al.*, 2001) and chimpanzees (Guidotti *et al.*, 1999). Furthermore, a recent elegant paper by Wieland *et al.* (2004) longitudinally analysed the activation of cellular genes in three experimentally infected chimpanzees. In all three animals, no cellular genes were activated within the liver during the lag phase of infection, confirming that intrahepatic activation of innate immunity did not affect initial HBV spread (Wieland *et al.*, 2004).

The causes of the delayed appearance of quantifiable levels of HBV proteins and HBV-DNA in the first weeks of infection are not clear. HBV might initially infect very few hepatocytes and spread with a relatively slow doubling time. Alternatively, we can speculate that immediately after infection, HBV does not reach the liver, but remains in other organs. Interestingly, longitudinal virological analysis of woodchuck hepatitis virus (WHV) infection showed that the initial site of WHV infection was not the liver but the bone marrow (Coffin & Michalak, 1999). However, the lymphotropism of WHV seems more pronounced, diffuse and with pathological importance than HBV (Coffin & Michalak, 1999; Lew & Michalak, 2001), and thus this possibility is attractive but still speculative in HBV infection. At the moment, we cannot correctly delineate the fate of HBV in the first 4 weeks after infection and subsequently we have ignored whether this apparent initial vanishing has an impact on the natural history of disease.

A further characteristic of HBV in relation to early host defence mechanisms resides in the lack of IFN- α and β production. HBV replication can be efficiently limited by α and β IFN (McClary *et al.*, 2000; Wieland *et al.*, 2000), but data on acutely infected chimpanzees suggest that such antiviral cytokines are not triggered by HBV replication (Wieland *et al.*, 2004). HBV might have evolved strategies to escape the initial antiviral defence mechanisms activated by the Toll-like receptor system. It has been proposed that because HBV replicates within nucleocapsid particles, viral replicative intermediates of single-stranded RNA or viral DNA, generally strong activators of type I IFN genes (Lund *et al.*, 2003; Heil *et al.*, 2004), are protected from cellular recognition (Wieland *et al.*, 2004).

A note of caution should follow the analysis of these data. Hepatitis, after HBV infection, is generally mild in chimpanzees compared with humans and it is possible that the inability to detect activation of genes related to innate immunity is a reflection of the mild profile of disease. Still, the striking difference between the early detection of type I IFN activation during early phases of HCV infection in chimpanzees (Bigger *et al.*, 2001; Su *et al.*, 2002) and its absence in HBV-infected animals is a further indication of the ability of HBV to sneak through the front line host defence mechanisms. Such early events are difficult to analyse during natural infection in humans. HBV-infected patients are mainly detected after onset of clinical symptoms (nausea and hycerus), which occur well after infection (10–12 weeks) (Webster *et al.*, 2000). Nevertheless, it is interesting to note that the lack of early symptoms in HBV-infected patients such as fever and malaise, which are characteristic of other human viral infections, constitutes indirect evidence of the defective type I IFN production during the early phases of HBV infection.

Triggering HBV immunity

Immediately after the exponential phase of HBV expansion, chimpanzees able to control the virus show a typical acute phase of disease with a robust activation of IFN- γ , TNF- α (Guidotti *et al.*, 1999) and many cellular genes linked to a T helper type 1 (Th1) type of cellular response (IFN- γ , IP-10 and Rantes) (Wieland *et al.*, 2004). It is possible that this initial host response to HBV is primarily sustained by NK and NK-T cells. Although we lack direct evidence for the role of NK and NK-T cells during natural HBV infection, the experimental data in animal models are consistent with the possibility that the initial burst of IFN- γ and the subsequent rapid inhibition of HBV could be mediated by these components of innate immunity. Activation of NK-T cells in the transgenic mouse model of HBV infection can inhibit virus replication through the production of IFN- γ (Kakimi *et al.*, 2000, 2001). Here, NK-T-cell activation was a consequence of α -galactoceramide stimulation rather than a response to the natural infection. However, recent results indicate that a population of non-classical NK-T cells can be directly activated when injected into mice expressing HBV antigens in the liver (Baron *et al.*, 2002). Thus, NK and NK-T cells could potentially be triggered during natural HBV infection, by the expression of stress signals either on infected hepatocytes or liver dendritic cells (Trobonjaca *et al.*, 2001) or possibly by direct recognition of viral components (Baron *et al.*, 2002).

Work on acutely infected chimpanzees is again providing the strongest evidence that NK and NK-T cells could be responsible for the initial control of HBV replication. In chimpanzees able to ultimately resolve the infection, a rapid drop in virus replication occurs in the presence of intrahepatic IFN- γ production, before the massive recruitment of T cells (Guidotti *et al.*, 1999). Despite the data in animal models, the only experimental evidence of NK-cell involvement in human HBV infection are represented by an analysis of NK-cell frequencies in patients studied during the incubation phase of acute hepatitis B. Here, increased numbers of circulating NK cells were concomitant with the peak of HBV replication, while, 2–4 weeks later, HBV-specific CD8 T cells appear when virus replication had already dropped (Webster *et al.*, 2000).

A different pattern is observed when patients or animal models infected with *Hepadnavirus* (WHV) develop chronicity. While virtually all patients that experience acute hepatitis B resolve the infection, development of chronicity is often associated with absent or mild symptoms of acute hepatitis. In line with these clinical observations, neonatally infected woodchucks that develop chronicity lack the large IFN- γ and TNF- α production observed in resolved animals (Cote *et al.*, 2000; Nakamura *et al.*, 2001; Menne *et al.*, 2002) and fail to develop an efficient antiviral-specific immune response.

Thus, activation of elements of innate immunity able to produce large quantities of IFN- γ seems to be a factor that determines the subsequent efficient induction of adaptive immunity and ultimately the outcome of HBV infection. What is at present unknown is what triggers this activation. Simple HBV quantity does not seem to be a separating criterion, since chronic patients ultimately reach HBV levels higher than resolved. Perhaps the kinetics of virus replication within the infected hepatocytes might directly influence the triggering of NK cells and the subsequent induction of an effective T-cell response (Bocharov *et al.*, 2004). What seems well established is that the differences in the adaptive immune response to HBV that characterize chronic and resolved patients are heavily influenced by the immunological events occurring during the initial phase of HBV replication.

Patterns of adaptive immunity

The adaptive immune response is comprised of a complex web of effector cell types, all of which play key roles in development of immunity to HBV. CD4 T cells, classically referred to as helper T cells, are robust producers of cytokines and are required for the efficient development of effector cytotoxic CD8 T-cells and B-cell antibody production. CD8 T cells go on to clear HBV-infected hepatocytes through cytolytic and non-cytolytic mechanisms (Guidotti & Chisari, 1996), reducing the levels of circulating virus, while B-cell antibody production neutralizes free viral particles and can prevent (re)infection (Alberti *et al.*, 1978; Grady *et al.*, 1978).

There are clear differences in the adaptive immunity of patients with established chronic or resolved HBV infection. HBV-specific CD4 and CD8 T-cell responses with a Th1 profile of cytokine production are detectable in the blood of subjects with a favourable outcome. These helper and cytotoxic responses are quantitatively stronger than those found in patients with

chronic infections, who are instead characterized by weaker or undetectable virus-specific T-cell responses (Ferrari *et al.*, 1990; Jung *et al.*, 1991, 1999; Penna *et al.*, 1991, 1996, 1997; Rehermann *et al.*, 1995b; Maini *et al.*, 1999; Sobao *et al.*, 2002; Webster *et al.*, 2004; Chang *et al.*, 2005). Whether the association between different outcomes of HBV infection and the vigour and breadth of the HBV-specific T-cell response has a causative effect has been difficult to demonstrate.

CD8 T-cell deletion experiments performed in HBV-infected chimpanzees have provided strong support for the concept that CD8 T cells are the main cellular subset responsible for viral clearance (Thimme *et al.*, 2003). Additional experiments in HBV patients or woodchucks demonstrate the importance of a coordinated helper and cytotoxic T-cell response in controlling hepadnavirus infection. In woodchucks, a reduced early expansion of virus-specific T cells was associated with virus persistence (Menne *et al.*, 2002), while in patients studied during the incubation phase of acute HBV infections, expansion of virus-specific IFN- γ^+ CD8 and CD4 T cells preceded complete virus clearance and was present only in subjects who controlled the infection (Webster *et al.*, 2000). The importance of coordinated activation of CD4 and CD8 T cells has been further demonstrated by the recent analysis of one HBV–HCV acutely co-infected patient who developed a chronic HBV infection. Longitudinal analysis of HBV-specific T-cell responses, from the time of infection to chronicity, shows the presence of a multi-specific CD8 T-cell response in the absence of a CD4 T-cell response (Urbani *et al.*, 2005). It is likely that the absence of CD4 help prevented the maturation of a functionally efficient CD8 T-cell response. Although, another possibility is that cytotoxic T cells were directed towards HBV regions without protective values or prone to viral mutations that can escape CTL recognition. Additional indirect evidence that CD4 and CD8 T-cell responses are accountable for the immunological control of HBV is represented by the association of particular HLA-class I and class II genetic profiles with resolution (Thursz *et al.*, 1997; Thio *et al.*, 2003).

Defining the characteristics of a T-cell response able to exert efficient *in vivo* antiviral function is a complex problem that has not been resolved in HBV infection. Often the concept of strong immunogenicity is associated with better protective values, but animal models have shown that immunodominance does not necessarily equate with protection (Gallimore *et al.*, 1998). The present knowledge about immunodominance and protective efficacy of different HBV proteins and epitopes will be discussed later.

Despite the cellular immune response being a major contributor to HBV clearance, humoral responses also play a role in controlling HBV. HBV clearance is associated with the production of anti-envelope antibodies (Alberti *et al.*, 1978) and sera with high levels of antiviral antibodies (specific for the viral envelope) that can control HBV infection (Grady *et al.*, 1978). Therefore, it is likely that the integrated activation of both the cellular and humoral arms of the adaptive immune response ultimately allows the host to control infection; the different components being so interconnected that the failure of one of them clearly affects the expansion and protective efficacy of the others. A lack of CD4 T cell help can impair CD8 T-cell activity and antibody production (Kalams & Walker, 1998), while the inability to mount a virus-

specific CD8 T-cell response results in a level of circulating virus that cannot be cleared by antibodies alone (Ciurea *et al.*, 2001).

Immunological hierarchy of HBV-specific CD4 and CD8 T-cell responses

Helper T-cell response. HBV-specific, HLA-class II-restricted CD4 T-cell responses have been characterized mainly in patients with self-limited acute hepatitis (Ferrari *et al.*, 1990; Jung *et al.*, 1991; Penna *et al.*, 1997). Multiple epitopes within the nucleocapsid protein are targeted by helper T cells of patients with self-limited hepatitis and immunodominant core epitopes have been identified within a sequence covering region 50–69, which can stimulate helper T cells in 90 % of patients tested, irrespective of HLA-class II profile (Ferrari *et al.*, 1991). The demonstration that increased core-specific CD4 responses are detectable during exacerbations of chronic hepatitis B, preceding HBeAg seroconversion (indicative of a reduced level of virus replication) (Tsai *et al.*, 1992; Rossol *et al.*, 1997), might represent an indication of the importance of the nucleocapsid-specific CD4 response in controlling HBV.

A different scenario is instead present for the envelope-specific CD4 T-cell response. In contrast to the immunogenicity of core antigen, the HBV envelope protein does not seem to expand an equally strong helper T-cell response during HBV infection (Ferrari *et al.*, 1990; Bocher *et al.*, 1999). The limited expansion of envelope-specific CD4 cells does not imply that envelope protein is a generally weak immunogen; on the contrary, the HBV envelope protein elicits strong helper T-cell responses in subjects vaccinated with a plasma-derived or recombinant form of this antigen (Celis *et al.*, 1988; Ferrari *et al.*, 1989; Bocher *et al.*, 1999). The differential immunogenicity of envelope antigens in vaccine recipients and in patients with natural infection suggests that differences in antigen presentation and/or the presence of 'natural' or synthetic adjuvant influences the immunogenicity of the responses in these two groups.

Even though most of the data have identified nucleocapsid-specific CD4 T cells as the dominant helper response correlating with HBV recovery, other aspects need to be considered. In particular, the helper T-cell response specific for the polymerase and X antigens have not been sufficiently investigated and only recently, polymerase epitopes able to elicit CD4 T-cell responses have been identified (Mizukoshi *et al.*, 2004). These polymerase epitopes were conserved among the different HBV genomes, bound to the most common HLA-DR and induced, in resolved acute hepatitis B patients, a helper T-cell response comparable to that detected against core peptides.

Cytotoxic T-cell response. Analysis of the HLA-class I-restricted CD8 T-cell response to HBV has been severely hampered by the inability of HBV to be propagated in cell culture (Chisari & Ferrari, 1995). The first definitive characterization of CD8 T cells specific for HBV derived from the understanding that the sequence of the processed viral antigens presented by HLA-class I molecules could be mimicked by synthetic peptides (Bertoletti *et al.*, 1991; Penna *et al.*, 1991). Thus, cytotoxic T cells specific for several viral epitopes within core (Bertoletti *et al.*, 1991;

Penna *et al.*, 1991; Missale *et al.*, 1993), envelope (Nayersina *et al.*, 1993), polymerase (Rehermann *et al.*, 1995b) and X (Hwang *et al.*, 2002) proteins of HBV were achieved using synthetic peptides, and not naturally processed epitopes, to expand memory cytotoxic T-lymphocyte (CTL) *in vitro*. These initial studies demonstrated that the magnitude of the HBV-specific CD8 response is stronger in self-limited than chronic infection (Bertoletti *et al.*, 1991; Penna *et al.*, 1991), that the CTL response persists decades after clinical recovery from acute infection (Rehermann *et al.*, 1996a) and that it can also be observed after resolution of chronicity (Rehermann *et al.*, 1996b). The majority of these studies have been carried out using peptides able to bind specifically to HLA-A2 molecules, with the result that a disproportionate number of known HBV epitopes are HLA-A2 restricted. However, HBV-specific cytotoxic epitopes restricted by different HLA-class I molecules (Missale *et al.*, 1993; Bertoni *et al.*, 1997; Sobao *et al.*, 2001; Thimme *et al.*, 2001) have also been identified.

The development of methods such as MHC/peptide tetramer staining, intracellular cytokine staining and Elispot, able to quantify virus-specific CD8 cells directly *ex vivo*, has permitted a more accurate analysis of HBV-specific CD8 T cells during the different phases of HBV infection. These data confirmed the quantitative differences between self-limited and chronic infection (Jung *et al.*, 1999; Maini *et al.*, 1999) and demonstrated that the quantity of HBV-specific CD8 T cells correlated with HBV control and not with liver damage (Maini *et al.*, 2000). This work also revealed that an epitope hierarchy exists within the HBV-specific CD8 T-cell responses that can be altered by viral persistence. Core 18–27-specific CD8 cells often represent the dominant response among the different A2-restricted epitopes tested in patients with acute hepatitis, but this is not absolute. In some patients, Pol 455–63-, Env 183–91- or Env 335–43-specific CD8 T cells were found to quantitatively dominate the CD8 T-cell response (Webster *et al.*, 2000, 2004).

The overall dominance of these three responses among the different HLA-A2-restricted epitopes within a patient is also maintained when immunodominance is defined as the most common responses among different patients (Bertoni *et al.*, 1997). The great majority of A2⁺ patients with self-limited hepatitis B recognize the HBc18–27, HBe183–91, HBe335–43 and HBp455–63 epitopes. The cause of immunodominance of these sequences is likely linked to their good binding affinity to the HLA-A2 molecule. A further possible explanation of the dominance of these HLA-A2-restricted CD8 responses is the finding that some HLA-class I epitopes are nested within helper T-cell epitopes. CD4-helper T cells are necessary for the maintenance of functional CD8 T cells and the covalent linkage between helper and cytotoxic epitopes has been shown to be important for the induction of CTL responses (Kalams & Walker, 1998). The well characterized, often immunodominant, HBc18–27 epitope overlaps with an HLA-class II-restricted epitope (Bertoletti *et al.*, 1997) and similar features have been described for new polymerase CD8 T-cell epitopes (Mizukoshi *et al.*, 2004). It must however be stressed that the overall hierarchy of CTL responses is still incomplete and there is no information available about competition among epitopes restricted by different HLA-class I alleles.

Despite these limitations, the detailed analysis of HBV-specific CD8 responses has led to important information regarding the potential impact of different CTL specificities on HBV

immunopathogenesis. Amino acid mutations within the core 18–27 region able to inhibit activation of the core 18–27-specific CD8 cells have been shown to occur in patients with chronic hepatitis B (Bertoletti *et al.*, 1994). In contrast, mutations within polymerase and envelope epitopes are rare (Rehermann *et al.*, 1995a) and cannot be identified even in chronic patients that demonstrate the presence of envelope and polymerase-specific CD8 cells (Webster *et al.*, 2004), suggesting that the antiviral pressure of the core 18–27-specific CD8 response is greater than the response against polymerase and envelope epitopes.

Longitudinal analysis of HLA-A2-restricted HBV-specific CD8 T cells in resolved and chronic hepatitis B patients have also revealed that the functional fate of epitope specificities differs markedly in chronic infection. Chronic hepatitis B is a heterogeneous disease that can vary greatly in the levels of virus replication, liver disease activity and humoral responses. The combined direct *ex vivo/in vitro* analysis of HBV-specific CD8 cells in chronic patients with different disease profiles demonstrated that core 18–27-specific CD8 T cells (often immunodominant in self-limited hepatitis) cannot be detected in the circulation (either directly *ex vivo* or after *in vitro* expansion) when HBV-DNA levels are $>10^7$ copies ml⁻¹. The inability to detect core 18–27-specific CD8 T cells within the circulatory compartment is not due to preferential intrahepatic localization; on the contrary, the frequency of core 18–27-specific CD8 T cells within the liver is inversely proportional to the level of HBV replication (Webster *et al.*, 2004).

Envelope and polymerase-specific CD8 T cells are the only specificities that can be demonstrated in chronic hepatitis B patients with concentrations of HBV-DNA $>10^7$ copies ml⁻¹ (Reignat *et al.*, 2002; Webster *et al.*, 2004). Their ability to persist in the face of high levels of HBV replication is associated with an apparent inability to exert antiviral function. Envelope-specific CD8 cells are characterized by an altered phenotype (tetramer/neg) (Reignat *et al.*, 2002), and their indifference to the dynamic fluctuations of HBV-DNA levels is suggestive of a tolerant state. The persistence of polymerase-specific CD8 T cells could be the result of the low quantity of polymerase epitopes expressed *in vivo* by infected hepatocytes, as suggested by results obtained in the transgenic mouse model of HBV infection (Kakimi *et al.*, 2002).

The collapse of HBV-specific T-cell response in chronic HBV patients

We have seen how the inability to control HBV infection and the establishment of chronicity lead to a state of relative collapse of virus-specific adaptive immunity. This state of HBV-specific T-cell tolerance is not absolute but appears to be regulated mainly by the quantity of HBV replication present in chronic hepatitis B patients. The impact of viral load on antiviral T-cell responses has been precisely characterized in animal models of viral infections (like LCMV), all of which show that sustained presence of viral antigens leads to a progressive functional decline of virus-specific CD8 responses (see Fig. 2) and ultimately leads to virus-specific T-cell deletion (Wherry *et al.*, 2003; Zhou *et al.*, 2004). Similarly, in HBV-infected patients, the frequency and function of circulating and intrahepatic HBV-specific CD8 T cells is inversely proportional to the level of HBV-DNA (Sobao *et al.*, 2002; Webster *et al.*, 2004).

HBeAg, a secretory form of the nucleocapsid antigen, is produced in large excess during HBV replication (Seeger & Mason, 2000). The tolerizing effect of HBeAg has been well characterized in mice (Milich *et al.*, 1990, 1998; Milich & Liang, 2003; Chen *et al.*, 2004, 2005) and likely contributes to the low level of core-specific T-cell responses present in HBeAg⁺ chronic patients. Clinical evidence supports the tolerogenic effect of HBeAg. Exacerbations of chronic hepatitis B are often associated with selection of HBV unable to produce HBeAg (Brunetto *et al.*, 1991). In addition, HBV replication is linked to the production of excessive amounts of the soluble form of HBsAg. Particles composed of only surface antigen are present in 10³–10⁶ fold excess over whole virions (Seeger & Mason, 2000). These particles are not infectious but the evolution of such impressive levels of synthetic effort by HBV may deliberately cause a state of low T-cell response and T-cell deletion.

Other factors, in addition to the quantity of viral antigens, have been suggested to explain the state of virus-specific T-cell collapse present in chronic hepatitis B patients (Fig. 3).

Dendritic cells. Dendritic cells represent a specialized antigen presenting cell population necessary for the induction of an adaptive immune response (Banchereau *et al.*, 2000). In relation to their crucial role in T-cell priming, functional alterations of dendritic cell populations could explain the state of T- and B-cell hypo-responsiveness present in chronic hepatitis B patients. However, even though dendritic cells are likely to be infected in animal models of hepadnavirus infection (Lew & Michalak, 2001) productive HBV replication has recently been excluded in chronic hepatitis B patients (Tavakoli *et al.*, 2004) and the stimulatory defects seem minimal (Wang *et al.*, 2001; Beckebaum *et al.*, 2002; Lohr *et al.*, 2002; van der Molen *et al.*, 2004). Thus, the role of dendritic cell functional impairment in maintaining a state of HBV-specific T-cell tolerance is, at the moment, controversial.

Regulatory T cells (CD4⁺ CD25⁺). Studies in numerous experimental models have provided evidence that a population of specialized T cells are able to regulate the immune response. These cells reside mainly within a minor population of CD4 cells that express the phenotypic marker CD25. They have been shown to suppress immunological responses against self (Sakaguchi, 2000) and foreign antigens (Suvas *et al.*, 2003) through suppressive cytokines or direct cell–cell contact; however, regulatory effects of CD4⁺ CD25⁺ cells have not been fully elucidated (Maloy & Powrie, 2001). It is possible that CD4⁺ CD25⁺ T cells are responsible for the weak HBV-specific T-cell response in chronic hepatitis B patients and may inhibit the expansion and function of HBV-specific CD8 T cells, precluding HBV clearance but also limiting immune mediated liver damage.

The impact of circulating CD4⁺ CD25⁺ T cells on HBV pathogenesis has recently been analysed. Increased frequencies of circulating regulatory cells in patients with chronic hepatitis B have been reported in some (Stoop *et al.*, 2005) but not in other studies (Franzese *et al.*, 2005). Depletion of CD4⁺ CD25⁺ cells increased the function of HBV-specific T cells (Franzese *et al.*, 2005; Stoop *et al.*, 2005), but such modulation was not HBV-specific and could be observed in patients with resolved HBV infection (Franzese *et al.*, 2005). This casts doubts on

the possible role of CD4⁺ CD25⁺ regulatory cells in the pathogenesis of chronic HBV infection. However, these studies were limited to the analysis of the CD4⁺ CD25⁺ cells present in the blood and a detailed analysis of the intrahepatic frequency and function of these cells is likely necessary to reveal their role. Furthermore, it is possible that a population of HBV-specific regulatory cells, different from the CD4⁺ CD25⁺ T-cell subset, analogous to the presence of IL-10 producing HCV-specific T cells (Accapezzato *et al.*, 2004), might be induced in chronic HBV infection (Hyodo *et al.*, 2004).

Liver environment. The immunological features of the liver might contribute to the maintenance of immunological tolerance present in chronic HBV infection. Data produced mainly in animal models have shown that CD8 T-cell induction, expansion, survival and antiviral function are altered following activation by antigens presented in the liver. In mice, hepatocyte priming of CD8 T cells preferentially induces tolerance and results in reduced CD8 T-cell clonal expansion (Bertolino *et al.*, 1998, 2001; Bowen *et al.*, 2004). It has also been demonstrated that apoptosis of activated CD8 T cells preferentially occurs in the liver (Crispe *et al.*, 2000). However, this idea is becoming somewhat controversial as recent work in mice has shown that rapid activation of naïve or effector CD8 T cells within the liver was followed by efficient expansion (Isogawa *et al.*, 2005; Klein & Crispe, 2006).

Hepatocytes express low levels of MHC-class I and require nearly 100-fold higher peptide concentrations compared with other antigen presenting cells to stimulate equivalent numbers of virus-specific CD8 T cells (A. J. Gehring and others, unpublished results). This would suggest that any pathogen infecting hepatocytes is less likely to be recognized by CD8 T cells and might allow HBV to avoid recognition when virus replication is reduced. Furthermore, hepatocytes, despite being extremely sensitive to cytokine-mediated control of virus replication (Guidotti & Chisari, 2001), are resistant to perforin/granzyme-mediated killing (Kafrouni *et al.*, 2001). Taken together, these features can contribute to weak HBV-specific T-cell responses and thus increase the chances of HBV to persist.

Concluding remarks

Increased knowledge of the virological and immunological events secondary to HBV infection allows us to define the mechanisms involved in viral clearance and persistence. Analysis of early events following HBV infection has revealed that HBV fails to activate early immunological responses, which are delayed until the exponential phase of replication (Webster *et al.*, 2000; Wieland *et al.*, 2004). Interestingly, the delayed kinetics of virus replication can explain why HBV vaccines are able to prevent infection, even if administered after exposure (Iwarson *et al.*, 1988). Even though virus-specific CD8 T cells play a major role in HBV clearance (Thimme *et al.*, 2003), coordinated activation of the different branches of adaptive immunity seems necessary to achieve viral control. When chronicity develops, diffuse defects of helper and cytotoxic T-cell responses are apparent and are likely to be maintained by the concerted action of high levels of viral antigens, the peculiar immunological features of the liver and perhaps by the contribution of regulatory cells or dendritic cell defects. The immunological defects are proportional to the level of HBV replication and attempts to restore HBV-specific immunity by inhibiting virus replication through antiviral treatment results in partial restoration (Boni *et al.*, 2001, 2003; Rigopoulou *et al.*, 2005) which, however, was inadequate to achieve viral clearance. It is likely that viral chronicity alters the repertoire of HBV-specific immunity to a level that makes its functional restoration very complex. Therapeutic vaccination combined with cytokines, use of dendritic cells or production of potent cytotoxic and helper T cells through T-cell receptor transfer are strategies under investigation to improve therapeutic chances to control this infection.

References

- Accapezzato, D., Francavilla, V., Paroli, M., Casciaro, M., Chircu, L. V., Cividini, A., Abrignani, S., Mondelli, M. U. & Barnaba, V. (2004).** Hepatic expansion of a virus-specific regulatory CD8⁺ T cell population in chronic hepatitis C virus infection. *J Clin Invest* **113**, 963–972.
- Alberti, A., Diana, S., Sculard, G. H., Eddleston, A. L. & Williams, R. (1978).** Detection of a new antibody system reacting with Dane particles in hepatitis B virus infection. *Br Med J* **2**, 1056–1058.
- Alberti, A., Chemello, L. & Benvegno, L. (1999).** Natural history of hepatitis C. *J Hepatol* **31**, S17–S24.
- Alexopoulou, L., Holt, A. C., Medzhitov, R. & Flavell, R. A. (2001).** Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* **413**, 732–738.
- Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B. & Palucka, K. (2000).** Immunobiology of dendritic cells. *Annu Rev Immunol* **18**, 767–811.
- Baron, J. L., Gardiner, L., Nishimura, S., Shinkai, K., Locksley, R. & Ganem, D. (2002).** Activation of a nonclassical NKT cell subset in a transgenic mouse model of hepatitis B virus infection. *Immunity* **16**, 583–594.
- Beckebaum, S., Cicinnati, V. R., Dworacki, G. & 8 other authors (2002).** Reduction in the circulating pDC1/pDC2 ratio and impaired function of ex vivo-generated DC1 in chronic hepatitis B infection. *Clin Immunol* **104**, 138–150.
- Berquist, K. R., Peterson, J. M., Murphy, B. L., Ebert, J. W., Maynard, J. E. & Purcell, R. H. (1975).** Hepatitis B antigens in serum and liver of chimpanzees acutely infected with hepatitis B virus. *Infect Immun* **12**, 602–605.
- Bertoletti, A. & Ferrari, C. (2003).** Kinetics of the immune response during HBV and HCV infection. *Hepatology* **38**, 4–13.
- Bertoletti, A., Ferrari, C., Fiaccadori, F., Penna, A., Margolskee, R., Schlicht, H. J., Fowler, P., Guilhot, S. & Chisari, F. V. (1991).** HLA class I-restricted human cytotoxic T cells recognize endogenously synthesized hepatitis B virus nucleocapsid antigen. *Proc Natl Acad Sci U S A* **88**, 10445–10449.
- Bertoletti, A., Costanzo, A., Chisari, F. V. & 7 other authors (1994).** Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. *J Exp Med* **180**, 933–943.
- Bertoletti, A., Southwood, S., Chesnut, R. & 7 other authors (1997).** Molecular features of the hepatitis B virus nucleocapsid T-cell epitope 18-27: interaction with HLA and T-cell receptor. *Hepatology* **26**, 1027–1034.
- Bertolino, P., Trescol-Biemont, M. C. & Rabourdin-Combe, C. (1998).** Hepatocytes induce functional activation of naive CD8⁺ T lymphocytes but fail to promote survival. *Eur J Immunol* **28**, 221–236.
- Bertolino, P., Bowen, D. G., McCaughan, G. W. & Fazekas de St Groth, B. (2001).** Antigen-specific primary activation of CD8⁺ T cells within the liver. *J Immunol* **166**, 5430–5438.

- Bertoni, R., Sidney, J., Fowler, P., Chesnut, R. W., Chisari, F. V. & Sette, A. (1997).** Human histocompatibility leukocyte antigen-binding supermotifs predict broadly cross-reactive cytotoxic T lymphocyte responses in patients with acute hepatitis. *J Clin Invest* **100**, 503–513.
- Bigger, C. B., Brasky, K. M. & Lanford, R. E. (2001).** DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. *J Virol* **75**, 7059–7066.
- Biron, C. A. (2001).** Interferons alpha and beta as immune regulators – a new look. *Immunity* **14**, 661–664.
- Bocharov, G., Ludewig, B., Bertoletti, A., Klenerman, P., Junt, T., Krebs, P., Luzyanina, T., Fraser, C. & Anderson, R. M. (2004).** Underwhelming the immune response: effect of slow virus growth on CD8⁺-T-lymphocyte responses. *J Virol* **78**, 2247–2254.
- Bocher, W. O., Herzog-Hauff, S., Schlaak, J., Meyer zum Buschenfeld, K. H. & Lohr, H. F. (1999).** Kinetics of hepatitis B surface antigen-specific immune responses in acute and chronic hepatitis B or after HBs vaccination: stimulation of the *in vitro* antibody response by interferon gamma. *Hepatology* **29**, 238–244.
- Boni, C., Penna, A., Ogg, G. S. & 9 other authors (2001).** Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. *Hepatology* **33**, 963–971.
- Boni, C., Penna, A., Bertoletti, A. & 10 other authors (2003).** Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. *J Hepatol* **39**, 595–605.
- Bowen, D. G., Zen, M., Holz, L., Davis, T., McCaughan, G. W. & Bertolino, P. (2004).** The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *J Clin Invest* **114**, 701–712.
- Brunetto, M. R., Giarin, M. M., Oliveri, F. & 8 other authors (1991).** Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. *Proc Natl Acad Sci U S A* **88**, 4186–4190.
- Celis, E., Ou, D. & Otvos, L., Jr (1988).** Recognition of hepatitis B surface antigen by human T lymphocytes. Proliferative and cytotoxic responses to a major antigenic determinant defined by synthetic peptides. *J Immunol* **140**, 1808–1815.
- Chang, J. J., Wightman, F., Bartholomeusz, A., Ayres, A., Kent, S. J., Sasadeusz, J. & Lewin, S. R. (2005).** Reduced hepatitis B virus (HBV)-specific CD4⁺ T-cell responses in human immunodeficiency virus type 1-HBV-coinfected individuals receiving HBV-active antiretroviral therapy. *J Virol* **79**, 3038–3051.
- Chen, M. T., Billaud, J. N., Sallberg, M., Guidotti, L. G., Chisari, F. V., Jones, J., Hughes, J. & Milich, D. R. (2004).** A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. *Proc Natl Acad Sci U S A* **101**, 14913–14918.
- Chen, M., Sallberg, M., Hughes, J., Jones, J., Guidotti, L. G., Chisari, F. V., Billaud, J. N. & Milich, D. R. (2005).** Immune tolerance split between hepatitis B virus precore and core proteins. *J Virol* **79**, 3016–3027.
- Chisari, F. V. & Ferrari, C. (1995).** Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* **13**, 29–60.

Ciurea, A., Hunziker, L., Klenerman, P., Hengartner, H. & Zinkernagel, R. M. (2001). Impairment of CD4⁺ T cell responses during chronic virus infection prevents neutralizing antibody responses against virus escape mutants. *J Exp Med* **193**, 297–305.

Coffin, C. S. & Michalak, T. I. (1999). Persistence of infectious hepadnavirus in the offspring of woodchuck mothers recovered from viral hepatitis. *J Clin Invest* **104**, 203–212.

Cote, P. J., Toshkov, I., Bellezza, C. & 9 other authors (2000). Temporal pathogenesis of experimental neonatal woodchuck hepatitis virus infection: increased initial viral load and decreased severity of acute hepatitis during the development of chronic viral infection. *Hepatology* **32**, 807–817.

Crispe, I. N., Dao, T., Klugewitz, K., Mehal, W. Z. & Metz, D. P. (2000). The liver as a site of T-cell apoptosis: graveyard, or killing field? *Immunol Rev* **174**, 47–62.

Ferrari, C., Penna, A., Bertoletti, A., Cavalli, A., Valli, A., Schianchi, C. & Fiaccadori, F. (1989). The preS1 antigen of hepatitis B virus is highly immunogenic at the T cell level in man. *J Clin Invest* **84**, 1314–1319.

Ferrari, C., Penna, A., Bertoletti, A., Valli, A., Antoni, A. D., Giuberti, T., Cavalli, A., Petit, M. A. & Fiaccadori, F. (1990). Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. *J Immunol* **145**, 3442–3449.

Ferrari, C., Bertoletti, A., Penna, A. & 8 other authors (1991). Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. *J Clin Invest* **88**, 214–222.

Fong, T. L., Di Bisceglie, A. M., Biswas, R., Waggoner, J. G., Wilson, L., Claggett, J. & Hoofnagle, J. H. (1994). High levels of viral replication during acute hepatitis B infection predict progression to chronicity. *J Med Virol* **43**, 155–158.

Franzese, O., Kennedy, P. T., Gehring, A. J., Gotto, J., Williams, R., Maini, M. K. & Bertoletti, A. (2005). Modulation of the CD8⁺-T-cell response by CD4⁺ CD25⁺ regulatory T cells in patients with hepatitis B virus infection. *J Virol* **79**, 3322–3328.

Gallimore, A., Dumrese, T., Hengartner, H., Zinkernagel, R. M. & Rammensee, H. G. (1998). Protective immunity does not correlate with the hierarchy of virus-specific cytotoxic T cell responses to naturally processed peptides. *J Exp Med* **187**, 1647–1657.

Ganem, D. & Prince, A. M. (2004). Hepatitis B virus infection – natural history and clinical consequences. *N Engl J Med* **350**, 1118–1129.

Grady, G. F., Lee, V. A., Prince, A. M. & 14 other authors (1978). Hepatitis B immune globulin for accidental exposures among medical personnel: final report of a multicenter controlled trial. *J Infect Dis* **138**, 625–638.

Guidotti, L. G. & Chisari, F. V. (1996). To kill or to cure: options in host defense against viral infection. *Curr Opin Immunol* **8**, 478–483.

Guidotti, L. G. & Chisari, F. V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* **19**, 65–91.

Guidotti, L. G., Rochford, R., Chung, J., Shapiro, M., Purcell, R. & Chisari, F. V. (1999). Viral clearance without destruction of infected cells during acute HBV infection. *Science* **284**, 825–829.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H. & Bauer, S. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* **303**, 1526–1529.

Hodgson, P. D. & Michalak, T. I. (2001). Augmented hepatic interferon gamma expression and T-cell influx characterize acute hepatitis progressing to recovery and residual lifelong virus persistence in experimental adult woodchuck hepatitis virus infection. *Hepatology* **34**, 1049–1059.

Hwang, Y. K., Kim, N. K., Park, J. M., Lee, K., Han, W. K., Kim, H. I. & Cheong, H. S. (2002). HLA-A2 1 restricted peptides from the HBx antigen induce specific CTL responses in vitro and in vivo. *Vaccine* **20**, 3770–3777.

Hyodo, N., Nakamura, I. & Imawari, M. (2004). Hepatitis B core antigen stimulates interleukin-10 secretion by both T cells and monocytes from peripheral blood of patients with chronic hepatitis B virus infection. *Clin Exp Immunol* **135**, 462–466.

Isogawa, M., Furuichi, Y. & Chisari, F. V. (2005). Oscillating CD8⁺ T cell effector functions after antigen recognition in the liver. *Immunity* **23**, 53–63.

Iwarson, S., Wahl, M., Ruttimann, E., Snoy, P., Seto, B. & Gerety, R. J. (1988). Successful postexposure vaccination against hepatitis B in chimpanzees. *J Med Virol* **25**, 433–439.

Jilbert, A. R., Wu, T. T., England, J. M., Hall, P. M., Carp, N. Z., O'Connell, A. P. & Mason, W. S. (1992). Rapid resolution of duck hepatitis B virus infections occurs after massive hepatocellular involvement. *J Virol* **66**, 1377–1388.

Jung, M. C., Spengler, U., Schraut, W. & 8 other authors (1991). Hepatitis B virus antigen-specific T-cell activation in patients with acute and chronic hepatitis B. *J Hepatol* **13**, 310–317.

Jung, M. C., Hartmann, B., Gerlach, J. T. & 9 other authors (1999). Virus-specific lymphokine production differs quantitatively but not qualitatively in acute and chronic hepatitis B infection. *Virology* **261**, 165–172.

Kafrouni, M. I., Brown, G. R. & Thiele, D. L. (2001). Virally infected hepatocytes are resistant to perforin-dependent CTL effector mechanisms. *J Immunol* **167**, 1566–1574.

Kajino, K., Jilbert, A. R., Saputelli, J., Aldrich, C. E., Cullen, J. & Mason, W. S. (1994). Woodchuck hepatitis virus infections: very rapid recovery after a prolonged viremia and infection of virtually every hepatocyte. *J Virol* **68**, 5792–5803.

Kakimi, K., Guidotti, L. G., Koezuka, Y. & Chisari, F. V. (2000). Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med* **192**, 921–930.

Kakimi, K., Lane, T. E., Chisari, F. V. & Guidotti, L. G. (2001). Cutting edge: inhibition of hepatitis B virus replication by activated NK T cells does not require inflammatory cell recruitment to the liver. *J Immunol* **167**, 6701–6705.

Kakimi, K., Isogawa, M., Chung, J., Sette, A. & Chisari, F. V. (2002). Immunogenicity and tolerogenicity of hepatitis B virus structural and nonstructural proteins: implications for immunotherapy of persistent viral infections. *J Virol* **76**, 8609–8620.

Kalams, S. A. & Walker, B. D. (1998). The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J Exp Med* **188**, 2199–2204.

Klein, I. & Crispe, I. N. (2006). Complete differentiation of CD8⁺ T cells activated locally within the transplanted liver. *J Exp Med* **203**, 437–447.

- Korba, B. E., Cote, P. J., Wells, F. V., Baldwin, B., Popper, H., Purcell, R. H., Tennant, B. C. & Gerin, J. L. (1989).** Natural history of woodchuck hepatitis virus infections during the course of experimental viral infection: molecular virologic features of the liver and lymphoid tissues. *J Virol* **63**, 1360–1370.
- Lew, Y. Y. & Michalak, T. I. (2001).** In vitro and in vivo infectivity and pathogenicity of the lymphoid cell-derived woodchuck hepatitis virus. *J Virol* **75**, 1770–1782.
- Lohr, H. F., Pingel, S., Bocher, W. O., Bernhard, H., Herzog-Hauff, S., Rose-John, S. & Galle, P. R. (2002).** Reduced virus specific T helper cell induction by autologous dendritic cells in patients with chronic hepatitis B – restoration by exogenous interleukin-12. *Clin Exp Immunol* **130**, 107–114.
- Lok, A. S. & McMahon, B. J. (2001).** Chronic hepatitis B. *Hepatology* **34**, 1225–1241.
- Lund, J., Sato, A., Akira, S., Medzhitov, R. & Iwasaki, A. (2003).** Toll-like receptor 9-mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med* **198**, 513–520.
- Maini, M. K., Boni, C., Ogg, G. S. & 10 other authors (1999).** Direct ex vivo analysis of hepatitis B virus-specific CD8⁺ T cells associated with the control of infection. *Gastroenterology* **117**, 1386–1396.
- Maini, M. K., Boni, C., Lee, C. K. & 12 other authors (2000).** The role of virus-specific CD8⁺ cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* **191**, 1269–1280.
- Maloy, K. J. & Powrie, F. (2001).** Regulatory T cells in the control of immune pathology. *Nat Immunol* **2**, 816–822.
- McClary, H., Koch, R., Chisari, F. V. & Guidotti, L. G. (2000).** Relative sensitivity of hepatitis B virus and other hepatotropic viruses to the antiviral effects of cytokines. *J Virol* **74**, 2255–2264.
- Menne, S., Roneker, C. A., Roggendorf, M., Gerin, J. L., Cote, P. J. & Tennant, B. C. (2002).** Deficiencies in the acute-phase cell-mediated immune response to viral antigens are associated with development of chronic woodchuck hepatitis virus infection following neonatal inoculation. *J Virol* **76**, 1769–1780.
- Milich, D. & Liang, T. J. (2003).** Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* **38**, 1075–1086.
- Milich, D. R., Jones, J. E., Hughes, J. L., Price, J., Raney, A. K. & McLachlan, A. (1990).** Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? *Proc Natl Acad Sci U S A* **87**, 6599–6603.
- Milich, D. R., Chen, M. K., Hughes, J. L. & Jones, J. E. (1998).** The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. *J Immunol* **160**, 2013–2021.
- Missale, G., Redeker, A., Person, J., Fowler, P., Guilhot, S., Schlicht, H. J., Ferrari, C. & Chisari, F. V. (1993).** HLA-A31- and HLA-Aw68-restricted cytotoxic T cell responses to a single hepatitis B virus nucleocapsid epitope during acute viral hepatitis. *J Exp Med* **177**, 751–762.
- Mizukoshi, E., Sidney, J., Livingston, B., Ghany, M., Hoofnagle, J. H., Sette, A. & Rehermann, B. (2004).** Cellular immune responses to the hepatitis B virus polymerase. *J Immunol* **173**, 5863–5871.

Moretta, L., Bottino, C., Pende, D., Vitale, M., Mingari, M. C. & Moretta, A. (2005). Human natural killer cells: molecular mechanisms controlling NK cell activation and tumor cell lysis. *Immunol Lett* **100**, 7–13.

Nakamura, I., Nupp, J. T., Cowlen, M., Hall, W. C., Tennant, B. C., Casey, J. L., Gerin, J. L. & Cote, P. J. (2001). Pathogenesis of experimental neonatal woodchuck hepatitis virus infection: chronicity as an outcome of infection is associated with a diminished acute hepatitis that is temporally deficient for the expression of interferon gamma and tumor necrosis factor-alpha messenger RNAs. *Hepatology* **33**, 439–447.

Nayersina, R., Fowler, P., Guilhot, S. & 7 other authors (1993). HLA A2 restricted cytotoxic T lymphocyte responses to multiple hepatitis B surface antigen epitopes during hepatitis B virus infection. *J Immunol* **150**, 4659–4671.

Ou, R., Zhou, S., Huang, L. & Moskophidis, D. (2001). Critical role for alpha/beta and gamma interferons in persistence of lymphocytic choriomeningitis virus by clonal exhaustion of cytotoxic T cells. *J Virol* **75**, 8407–8423.

Penna, A., Chisari, F. V., Bertoletti, A., Missale, G., Fowler, P., Giuberti, T., Fiaccadori, F. & Ferrari, C. (1991). Cytotoxic T lymphocytes recognize an HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. *J Exp Med* **174**, 1565–1570.

Penna, A., Artini, M., Cavalli, A. & 8 other authors (1996). Long-lasting memory T cell responses following self-limited acute hepatitis B. *J Clin Invest* **98**, 1185–1194.

Penna, A., Del Prete, G., Cavalli, A. & 8 other authors (1997). Predominant T-helper 1 cytokine profile of hepatitis B virus nucleocapsid-specific T cells in acute self-limited hepatitis B. *Hepatology* **25**, 1022–1027.

Rehermann, B., Pasquinelli, C., Mosier, S. M. & Chisari, F. V. (1995a). Hepatitis B virus (HBV) sequence variation of cytotoxic T lymphocyte epitopes is not common in patients with chronic HBV infection. *J Clin Invest* **96**, 1527–1534.

Rehermann, B., Fowler, P., Sidney, J., Person, J., Redeker, A., Brown, M., Moss, B., Sette, A. & Chisari, F. V. (1995b). The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. *J Exp Med* **181**, 1047–1058.

Rehermann, B., Ferrari, C., Pasquinelli, C. & Chisari, F. V. (1996a). The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* **2**, 1104–1108.

Rehermann, B., Lau, D., Hoofnagle, J. H. & Chisari, F. V. (1996b). Cytotoxic T lymphocyte responsiveness after resolution of chronic hepatitis B virus infection. *J Clin Invest* **97**, 1655–1665.

Reignat, S., Webster, G. J., Brown, D. & 7 other authors (2002). Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. *J Exp Med* **195**, 1089–1101.

Rigopoulou, E. I., Suri, D., Chokshi, S., Mullerova, I., Rice, S., Tedder, R. S., Williams, R. & Naoumov, N. V. (2005). Lamivudine plus interleukin-12 combination therapy in chronic hepatitis B: antiviral and immunological activity. *Hepatology* **42**, 1028–1036.

Rossol, S., Marinos, G., Carucci, P., Singer, M. V., Williams, R. & Naoumov, N. V. (1997). Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. *J Clin Invest* **99**, 3025–3033.

Sakaguchi, S. (2000). Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* **101**, 455–458.

Seeger, C. & Mason, W. S. (2000). Hepatitis B virus biology. *Microbiol Mol Biol Rev* **64**, 51–68.

Sobao, Y., Sugi, K., Tomiyama, H. & 7 other authors (2001). Identification of hepatitis B virus-specific CTL epitopes presented by HLA-A*2402, the most common HLA class I allele in East Asia. *J Hepatol* **34**, 922–929.

Sobao, Y., Tomiyama, H., Sugi, K. & 7 other authors (2002). The role of hepatitis B virus-specific memory CD8 T cells in the control of viral replication. *J Hepatol* **36**, 105–115.

Stoop, J. N., van der Molen, R. G., Baan, C. C., van der Laan, L. J., Kuipers, E. J., Kusters, J. G. & Janssen, H. L. (2005). Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* **41**, 771–778.

Su, A. I., Pezacki, J. P., Wodicka, L. & 8 other authors (2002). Genomic analysis of the host response to hepatitis C virus infection. *Proc Natl Acad Sci U S A* **99**, 15669–15674.

Suvas, S., Kumaraguru, U., Pack, C. D., Lee, S. & Rouse, B. T. (2003). CD4⁺CD25⁺ T cells regulate virus-specific primary and memory CD8⁺ T cell responses. *J Exp Med* **198**, 889–901.

Tavakoli, S., Schwerin, W., Rohwer, A. & 9 other authors (2004). Phenotype and function of monocyte derived dendritic cells in chronic hepatitis B virus infection. *J Gen Virol* **85**, 2829–2836.

Thimme, R., Chang, K. M., Pemberton, J., Sette, A. & Chisari, F. V. (2001). Degenerate immunogenicity of an HLA-A2-restricted hepatitis B virus nucleocapsid cytotoxic T-lymphocyte epitope that is also presented by HLA-B51. *J Virol* **75**, 3984–3987.

Thimme, R., Bukh, J., Spangenberg, H. C., Wieland, S., Pemberton, J., Steiger, C., Govindarajan, S., Purcell, R. H. & Chisari, F. V. (2002). Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci U S A* **99**, 15661–15668.

Thimme, R., Wieland, S., Steiger, C., Ghayeb, J., Reimann, K. A., Purcell, R. H. & Chisari, F. V. (2003). CD8⁺ T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol* **77**, 68–76.

Thio, C. L., Thomas, D. L., Karacki, P. & 10 other authors (2003). Comprehensive analysis of class I and class II HLA antigens and chronic hepatitis B virus infection. *J Virol* **77**, 12083–12087.

Thursz, M. R., Thomas, H. C., Greenwood, B. M. & Hill, A. V. (1997). Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nat Genet* **17**, 11–12.

Trobonjaca, Z., Leithauser, F., Moller, P., Schirmbeck, R. & Reimann, J. (2001). Activating immunity in the liver. I. Liver dendritic cells (but not hepatocytes) are potent activators of IFN- γ release by liver NKT cells. *J Immunol* **167**, 1413–1422.

Tsai, S. L., Chen, P. J., Lai, M. Y., Yang, P. M., Sung, J. L., Huang, J. H., Hwang, L. H., Chang, T. H. & Chen, D. S. (1992). Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. *J Clin Invest* **89**, 87–96.

- Urbani, S., Boni, C., Amadei, B., Fisicaro, P., Cerioni, S., Valli, M. A., Missale, G. & Ferrari, C. (2005).** Acute phase HBV-specific T cell responses associated with HBV persistence after HBV/HCV coinfection. *Hepatology* **41**, 826–831.
- van der Molen, R. G., Sprengers, D., Binda, R. S., de Jong, E. C., Niesters, H. G., Kusters, J. G., Kwekkeboom, J. & Janssen, H. L. (2004).** Functional impairment of myeloid and plasmacytoid dendritic cells of patients with chronic hepatitis B. *Hepatology* **40**, 738–746.
- Wang, F. S., Xing, L. H., Liu, M. X., Zhu, C. L., Liu, H. G., Wang, H. F. & Lei, Z. Y. (2001).** Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* **7**, 537–541.
- Webster, G. J., Reignat, S., Maini, M. K. & 9 other authors (2000).** Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* **32**, 1117–1124.
- Webster, G. J., Reignat, S., Brown, D., Ogg, G. S., Jones, L., Seneviratne, S. L., Williams, R., Dusheiko, G. & Bertoletti, A. (2004).** Longitudinal analysis of CD8⁺ T cells specific for structural and nonstructural hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. *J Virol* **78**, 5707–5719.
- Whalley, S. A., Murray, J. M., Brown, D., Webster, G. J., Emery, V. C., Dusheiko, G. M. & Perelson, A. S. (2001).** Kinetics of acute hepatitis B virus infection in humans. *J Exp Med* **193**, 847–854.
- Wherry, E. J., Blattman, J. N., Murali-Krishna, K., van der Most, R. & Ahmed, R. (2003).** Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J Virol* **77**, 4911–4927.
- Wieland, S. F. & Chisari, F. V. (2005).** Stealth and cunning: hepatitis B and hepatitis C viruses. *J Virol* **79**, 9369–9380.
- Wieland, S. F., Guidotti, L. G. & Chisari, F. V. (2000).** Intrahepatic induction of alpha/beta interferon eliminates viral RNA-containing capsids in hepatitis B virus transgenic mice. *J Virol* **74**, 4165–4173.
- Wieland, S., Thimme, R., Purcell, R. H. & Chisari, F. V. (2004).** Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* **101**, 6669–6674.
- Zhou, S., Ou, R., Huang, L., Price, G. E. & Moskophidis, D. (2004).** Differential tissue-specific regulation of antiviral CD8⁺ T-cell immune responses during chronic viral infection. *J Virol* **78**, 3578–3600.

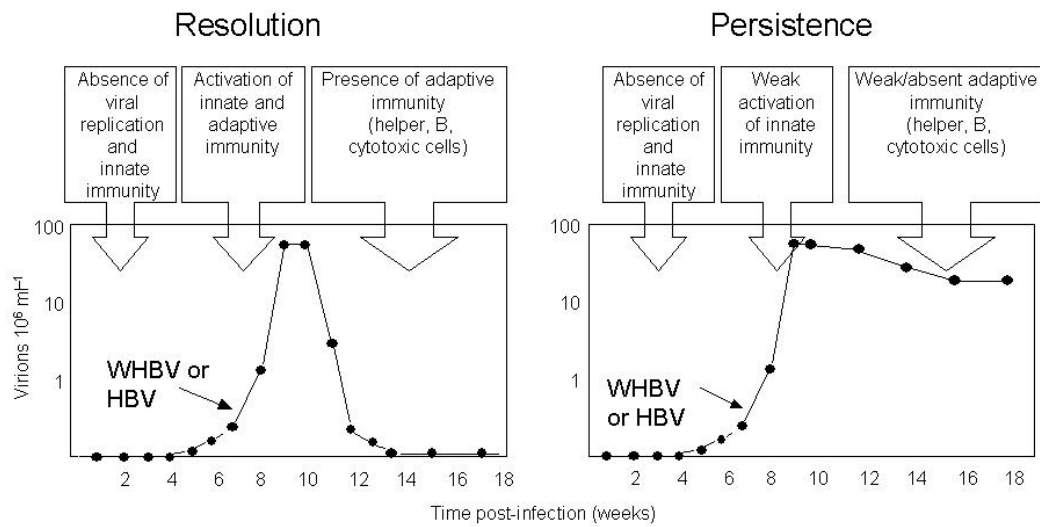


Fig. 1. Coordinate activation of innate and adaptive response is necessary for HBV control. Data from: Guidotti *et al.* (1999); Thimme *et al.* (2003); Nakamura *et al.* (2001); Menne *et al.* (2002); and Cote *et al.* (2000).

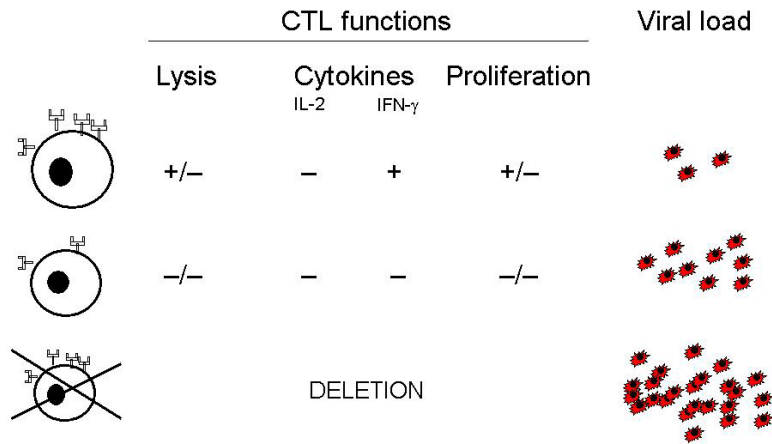


Fig. 2. Correlation of T-cell defects with virus replication levels. LCMV chronic infection/animal model (Wherry *et al.*, 2003).

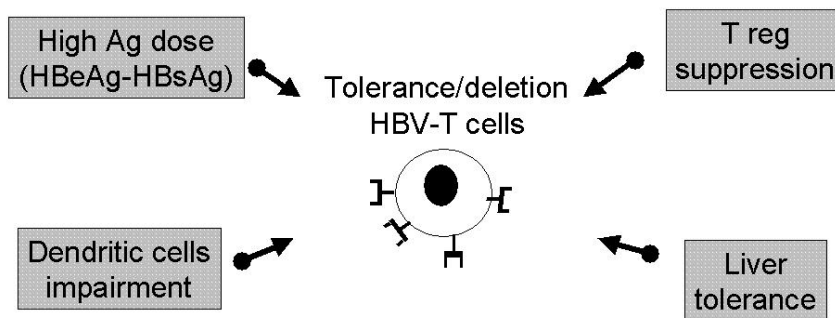


Fig. 3. HBV-specific T-cell tolerance. Causes.