The immune response during hepatitis B virus infection

Antonio Bertoletti and Adam J. Gehring

The UCL Institute of Hepatology, University College of London, 69–75 Chenies Mews, London WC1E 6HX, 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 $10^{6}$–$10^{10}$ 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

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**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).
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-galactosceramide 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 T cell 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 HBsAg 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 have polymerase epitopes able to elicit CD4 T-cell responses been identified (Mizukoshi et al., 1991).
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, cytotxic 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-lymphocytes (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 response 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 10^-5-10^-6-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).

**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.
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 of 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.

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