Pathogenesis of hepatitis B virus infection and potential for new therapies

Chronic hepatitis B virus infection leads to about 1 million deaths per year, making it one of the top ten causes of death worldwide. An improved understanding of defective antiviral responses in chronic hepatitis B virus infection could allow specific immunotherapeutic interventions, enhancing the immune response to hepatitis B virus.

Despite the availability of a prophylactic hepatitis B virus vaccine (Lavanchy, 2004), persistent infection with hepatitis B virus remains an important global public health problem, affecting an estimated 400 million people worldwide. The major complications of chronic hepatitis B virus infection include progression to cirrhosis, hepatic decompensation and hepatocellular carcinoma (Liaw and Chu, 2009). Although the UK has a low prevalence of chronic hepatitis B virus infection, in recent years the number of infected individuals has doubled, mainly as a result of increasing migration patterns from countries of intermediate or high hepatitis B virus prevalence. Despite this, the UK has not adopted a universal vaccination programme for hepatitis B virus.

The outcome of infection with hepatitis B virus depends on age and host–virus interactions. In patients with chronic hepatitis B virus infection, both adaptive and innate arms of immunity are skewed towards an ineffective response (Bertoletti and Ferrari, 2011), with profound depletion of T cell responses being the most characteristic feature in these patients. Although the introduction of new antiviral agents with a higher genetic barrier to resistance has improved the treatment of chronic hepatitis B virus infection, sustained off-treatment responses or durable reconstitution of antiviral immunity are still rarely achieved (Papatheodoridis et al, 2008); a large proportion of patients are therefore confined to long-term antiviral treatment with the problems of cost, toxicity and emergence of viral resistance.

A finite duration of treatment with pegylated interferon-α has a number of theoretical advantages, including the absence of resistance and immunomodulating effects potentiating sustained virological responses off treatment, but is often limited by toxicity (Papatheodoridis et al, 2008). The need to combine antivirals with new immunotherapeutic approaches has therefore been widely acknowledged. This review discusses different aspects of the virus–host interaction and highlights some of the key molecular defects of antiviral immunity which may formulate the basis of future novel therapies.

Hepatitis B virus: key features

Although a detailed overview of the molecular biology of hepatitis B virus is beyond the scope of this review, several key features have been implicated in the pathogenesis and clinical outcome of hepatitis B virus infection. Classified as a hepatotropic DNA virus, it replicates in the liver where almost all hepatocytes can become infected with hepatitis B virus. The virus itself is not directly cytopathic and liver damage is thought to be immune-mediated. During the replication cycle of hepatitis B virus, the partially double-stranded DNA genome is converted into a covalently closed circular DNA that resides within the host nucleus, establishing a lifelong hepatitis B virus reservoir (Locarnini and Zoulim, 2010). Owing to the persistence of this transcriptional template in hepatocytes, some inactive carriers can develop hepatitis B virus reactivation with either the wild type and reversion to e-antigen (eAg) positivity or with hepatitis B virus variants with mutations (Fattovich et al, 2008). One of the main challenges of antiviral treatments is to eradicate or limit the formation of covalently closed circular DNA.

Unlike all known mammalian DNA viruses, hepatitis B virus uses an RNA intermediate and is dependent on virus-encoded reverse transcriptase. Replication by reverse transcription is, however, error-prone and responsible for the emergence of a heterogenous viral population (including those containing mutations affecting the production of eAg associated with unique clinical manifestations as well as mutations conferring resistance to antiviral therapy) (Locarnini and Zoulim, 2010). Mutational escape to bypass immune detection as an evasion strategy is thought to be relatively uncommon during chronic hepatitis B virus infection, where there is a characteristically weak CD8 T cell response and low selection pressure on the virus, in contrast to hepatitis C virus infection (Rehermann and Nascimbeni, 2005).

Immune tolerance by viral antigens

Another unique feature of hepatitis B virus is the ability of an infected cell to secrete large amounts of circulating HBsAg and HBeAg which do not contain the hepatitis B virus genome. These antigens are secreted in large excess

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of complete virions, and are postulated to have an immunomodulatory function that protects the virus against immune attack (Milich and Liang, 2003; Wieland and Chisari, 2005). In particular HBeAg, the secretory form of the core antigen, which does not have a role in viral replication, has been proposed to induce tolerance in utero that may predispose neonates born to hepatitis B virus-infected mothers to develop persistent infection (Milich et al, 1990). In addition, the HBx protein has the potential when overexpressed to inhibit the processing and presentation of viral antigen (Wieland and Chisari, 2005). These key viral factors may favour persistence and perpetuate tolerance of the various effector arms of the immune response.

Host factors

The immune response

The immune system has the inherent capacity to combat hepatitis B virus infection, exemplified by the fact that the majority of infected adults successfully control acute infection. The potential for immune control in the setting of established chronic infection has been highlighted by resolution of chronic hepatitis B virus infection in recipients following bone marrow transplantation from immune donors (Ilan et al, 1993). Given that hepatitis B virus has a host range limited to humans and chimpanzees, elucidating the various immunological phenomena during infection has been challenging.

Innate arm

Innate immunity is an important first line of defence against early infection, with type I interferons having both a direct antiviral effect and helping to shape the development of adaptive responses. Studies from the chimpanzee model have, however, shown a lack of induction of type I interferon genes in the liver during the entry and expansion phase of hepatitis B virus (Wieland et al, 2004). In marked contrast with other infections such as human immunodeficiency virus, human cytomegalovirus or dengue virus, hepatitis B virus is characterized by delayed propagation following infection and a lack of early clinical manifestations of an early viral infection, constituting indirect evidence of the defective type I interferon production (Bertoletti et al, 2010). These findings and observations have led to the widely held belief that hepatitis B virus is a stealth virus which can replicate under the radar of innate immunity.

More recent work from a cohort of patients sampled in the early pre-clinical phase of hepatitis B virus and followed up to resolution of infection has highlighted the role of the immunosuppressive cytokine interleukin-10 (IL-10) in actively suppressing early innate effector responses (Dunn et al, 2009). Even though induction of IL-10 in this setting does not prevent eventual viral control in the majority of patients with acute infection, it may provide an opportunity for viral escape. In persistent infection with hepatitis B virus, ongoing induction of IL-10 attenuates the action of NK cells, a crucial component of the innate arm of immune response, thereby potentially contributing to tolerance and viral persistence (Peppa et al, 2010). In chronic infection, in addition to NK cell defects, alterations of dendritic cell populations (a specialized antigen-presenting cell population) have been described, further impairing effective cross-talk and priming of adaptive immune responses (van der Molen et al, 2004). A better understanding of the sensors and effectors of innate immunity relevant to hepatitis B virus may therefore have important therapeutic implications.

The adaptive immune response: the collapse of the T cell response in chronic hepatitis B virus infection

A lot of research has been focused on CD8 T cells, a crucial component of immunity to non-cytopathic viruses; their critical role in hepatitis B virus has been demonstrated in chimpanzee infection and supported by findings from human studies (Maini et al, 2000; Thimme et al, 2003). In patients with self-limiting hepatitis B virus infection there is a vigorous and multi-specific T cell response whereas the hallmark of chronic infection is a weak and transient T cell response.

Why do T cells fail to adequately control hepatitis B virus during chronic infection? In addition to having T cells of narrower specificity, chronically infected patients have characteristically diminished numbers of virus-specific T cells (Rehermann et al, 1995). This depletion of virus-specific T cells is partially attributed to the upregulation of pro-apoptotic molecules (Bcl2-interacting mediator) and their enhanced vulnerability to death (Lopes et al, 2008). In patients with poor control of viral replication, the few remaining virus-specific T cells identified are unable to mount a robust immune response and have reduced proliferative potential (Maini and Schurich, 2010). They have therefore been termed dysfunctional or exhausted.

Animal models of persistent viral infections such as lymphocytic choriomeningitis virus have characterized the impact of viral load and continuous antigenic stimulation on progressive functional decline of virus-specific T cells (Wherry et al, 2003). This scenario may be applicable in chronic hepatitis B virus infection, where T cells are exposed to decades of high-level viral replication; circulating intrahepatic hepatitis B virus-specific CD8 T cells are inversely proportional to hepatitis B virus DNA levels (Webster et al, 2004). Therefore the quantitative and qualitative deficiencies observed in hepatitis B virus-specific CD8 may be partially attributable to sustained consumption following repeated cycles of T cell activation.

Exposure to viral antigens could further contribute to T cell dysfunction. This notion is supported by the fact that production of the sub-viral particles is not switched off by antiviral drugs even with successful suppression of viral replication. Central to the observed T cell exhaustion in chronic hepatitis B virus infection is the upregulation of co-inhibitory molecules such as PD-1 and CTLA-4 (Boni et al, 2007; Schurich et al, 2011). Although therapeutic blockade of these receptors may constitute a promising approach, co-expression of additional inhibitory molecules on T cell populations illustrates the complexity of cooperation and redundancy of the various pathways. Therefore, a tailored strategy of targeting multiple co-inhibitory signals and promoting co-stimulation may help balance the signals regulating T cell antiviral function.

In order to better understand the mechanisms of exhaustion one must appreciate the unique immunological features of the liver environment. Constantly exposed to innocuous gastrointestinal antigens, the liver is naturally an immunotolerant site (Protzer et al, 2012). T cell exhaustion may be perpetuated in the liver by inefficient or tolerogenic activation by the potential antigen-presenting cells such as hepatocytes and liver sinusoidal epithelial cells (Protzer et al, 2012). Priming of CD4 T cells in the liver leads to poor production of Th1 cytokines (Knolle et al, 1999; Protzer et al, 2012), while priming of CD8 T cells induces only transient cell activation and eventual CD8 T cell attrition by apoptosis (Protzer et al, 2012). Moreover, deficiencies in the nutrient milieu in the liver, such as depletion of arginine (Das et al, 2008) and high levels of the immunosuppressive cytokines IL-10 and transforming growth factor-beta (TGF-β), can further impair the maintenance of robust T cell responses. Immunotherapeutic targeting of IL-10 and TGF-β in chronic hepatitis B virus infection remains a possibility, with the caveat that these cytokines regulate a critical balance between preventing pathogen clearance and improving immunopathology. Although the precise role of regulatory populations in chronic hepatitis B virus infection remains controversial, some studies have suggested that they can also hamper the ability of T cells to expand and survive (Manigold and Racanelli, 2007).

CD4 T cells are particularly important in priming CD8 T cells and CD4 T cell activation is required to arm CD8 T cell responses. However, mirroring the CD8 T cells, chronically infected patients also have a narrower repertoire of virus-specific CD4 T cells (with poorer function) compared to resolved patients (Bertoletti and Ferrari, 2011).

Pathogenesis of liver damage

The immune response in chronic hepatitis B virus infection is also responsible for immunopathology and liver inflammation. It was initially hypothesized that the cytolytic function of virus-specific CD8+ cytotoxic T lymphocytes was solely responsible for the death of hepatitis B virus-infected hepatocytes. Elegant experiments in the hepatitis B virus transgenic mouse model demonstrated a role for the non-specific infiltrate recruited in the liver at the time of peak inflammation (Ando et al, 1993). This was subsequently confirmed in human chronic hepatitis B virus infection, where comparable numbers of intrahepatic virus-specific CD8 T cells were found to be associated with either protection or pathology (Maini et al, 2000). In patients with active disease and biochemical evidence of liver inflammation there was an influx of non-antigen specific lymphocytes highlighting their importance in the pathogenesis of liver damage (Maini et al, 2000). Studies suggest that NK cells expressing a death ligand, TRAIL, can contribute to hepatocyte damage during disease flares in eAg- chronic hepatitis B virus-infected patients (Dunn et al, 2007). This pathway is switched on following cytokine fluctuations produced during active hepatitis B virus infection (Dunn et al, 2007). These data lend support to the idea that future immunotherapeutic strategies should aim to restore T cell defects by promoting non-cytolytic clearance of the virus, while blocking potential pathways of damage.

Conclusions

A question remaining is how can the immune response be safely harnessed in chronic hepatitis B virus infection to promote viral clearance?

Given that interferon-α therapy is one of the most successful current treatments, augmentation of innate immunity may be a useful future strategy. However, further understanding of the immunomodulatory effects of interferon-α is necessary to tailor more effective approaches. Alternative approaches of targeting innate responses include directing interferon-α towards infected hepatocytes with TCR-like monoclonal antibodies (Bertoletti and Ferrari, 2011). This would allow for a more localized effect and avoid generalized systemic symptoms often encountered with current treatment. Substituting interferon alpha with interferon lambda, a type III interferon which is preferentially produced in the liver, is an attractive option but the potential role of these cytokines in hepatitis B virus control merit further study (Bertoletti et al, 2010). The lack of in-vivo detection of hepatitis B virus raises the possibility of stimulating innate recognition with agonists for pattern recognition receptors (Bertoletti and Ferrari, 2011). The potential role of innate components in mediating liver pathology highlights the need for carefully timed intervention.

Therapeutic vaccination has been used in chronic hepatitis B virus infection with the aim of restoring the antiviral T cell response (Bertoletti and Gehring, 2009). However, these interventions have been of limited success, highlighting the degree of immune collapse in chronic infection. Overcoming tolerance in chronic hepatitis B virus infection is an attractive option which needs to be carefully weighed against the risk of a generalized reconstitution of T cells and exuberant immune responses. Thus future therapies should aim to first reduce viral load and then boost hepatitis B virus-specific T cells using a multifaceted approach of targeted immunotherapeutic intervention with a therapeutic hepatitis B virus vaccine. Such an approach has been successfully used in mouse models of chronic viral infections (Ha et al, 2008). Chimeric antigen receptors and T cell receptor gene transfer are exciting alternative strategies, attempting to redirect existing T cells against hepatitis B virus (Bertoletti and Gehring, 2009).

Although a number of recent advances have shed light on the mechanisms of immune dysfunction in chronic hepatitis B virus infection, further work is still required. A more comprehensive knowledge of the immunological phases of chronic hepatitis B virus infection, potential pathways and triggers of liver pathology and molecular mechanisms underlying viral persistence will help inform future therapeutic strategies. **BJHM**

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KEY POINTS

- The outcome of infection with hepatitis B virus depends on age and host–virus interactions.
- The immune response in chronic hepatitis B virus infection is also responsible for immunopathology and liver inflammation.
- T cells fail to adequately control hepatitis B virus during chronic infection.
- An improved understanding of the defective antiviral responses in chronic hepatitis B virus infection could pave the way towards targeting specific immunotherapeutic interventions enhancing the natural ability of the immune response to control hepatitis B virus.