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REVIEW

The role of tumour necrosis factor in hepatitis B infection: Jekyll and Hyde

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Chronic hepatitis B (CHB) is a major health problem worldwide and is associated with significant long-term morbidity and mortality. The hepatitis B virus (HBV) is a hepatotropic virus that is capable of integrating in the host nucleus permanently resulting in lifelong infection. To date, there is no definitive cure for HBV, as our current treatments cannot eradicate the viral reservoir that has integrated in the liver. Elucidating the immunopathogenesis is key to finding a therapeutic target for HBV as the virus is not in itself cytopathic but the immune response to the virus causes the majority of the cellular injury. In most cases, the virus reaches a state of equilibrium with low viral replication constrained by host immunity. Multiple cytokines have been implicated in the pathogenesis of CHB. Tumor necrosis factor (TNF) has emerged as a key player; on one hand it can facilitate immune-mediated virological control but on the other hand it can cause collateral hepatocyte damage, cirrhosis and possibly promote hepatocellular carcinoma. In this review, we discuss the current understanding of the immunopathogenesis of HBV, focusing on TNF and whether it can be harnessed in therapeutic strategies to cure HBV infection.
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INTRODUCTION

Hepatitis B virus (HBV) has infected more than 2 billion people globally and of those, an estimated 350 million people have become chronic carriers.[1–3] Chronic hepatitis B (CHB) infection is associated with significant mortality and morbidity. It is a major risk factor for liver cirrhosis and the leading cause of hepatocellular carcinoma.[4] Up to 40% of patients with CHB develop serious liver disease, leading to 1.2 million deaths per year[5] making HBV the 10th leading cause of death worldwide.[3]

CHB is endemic in South East Asia, China, sub-Saharan Africa, Micronesia and Polynesia, and the indigenous populations of Alaska, Northern Canada, Greenland, Australia and New Zealand. More than 7% of the population is chronically infected in these high prevalence regions[6] and ~45% of the global population lives in an area of high prevalence.

Most infections are acquired early in childhood and the risk of chronicity is inversely related to age at the time of infection. Perinatal transmission leads to chronicity in >90% cases. In contrast, infections acquired later in life tend to have a symptomatic acute phase but only a small proportion (<5%) of immunocompetent patients develop CHB.[7,8] There is a safe and effective vaccine for HBV. The World Health Organisation recommends universal immunization of neonates. However, in low-income countries, where HBV is endemic, access to vaccination is sub-optimal and CHB continues to have a significant burden of disease.[9]

To date, there is no definitive cure for HBV. Pegylated interferon and nucleos(t)ide analogs including Lamivudine, Adefovir, Entecavir and Tenofovir are currently used in the clinical setting but rates of viral clearance are poor. These agents can successfully suppress viral replication cannot eradicate virus permanently. This is because, unlike other hepatitis viruses, the HBV transcriptional template persists in the nucleus of infected cells as a covalently closed circular DNA (cccDNA) mini chromosome. In addition, subgenomic HBV DNA can integrate into the host chromosomes. These HBV elements are persistent and they are virtually impossible to eradicate unless the hepatocyte is killed.

The pathogenesis of HBV is complex and the host–virus interactions have not been entirely elucidated. The immunological response of the host is central in HBV infection as it determines both the natural history and clinical outcomes of disease.

Multiple immune mediators are implicated in the pathogenesis of CHB, and the role of many of these mediators is unclear. Of the myriad of cytokines and chemokines involved the Tumour Necrosis Factor (TNF) has been recognized as one of the key players. TNF is of great clinical and scientific interest as it is implicated in many autoimmune and inflammatory diseases. It is clear that excessive TNF can promote these types of diseases but the advent of anti-TNF

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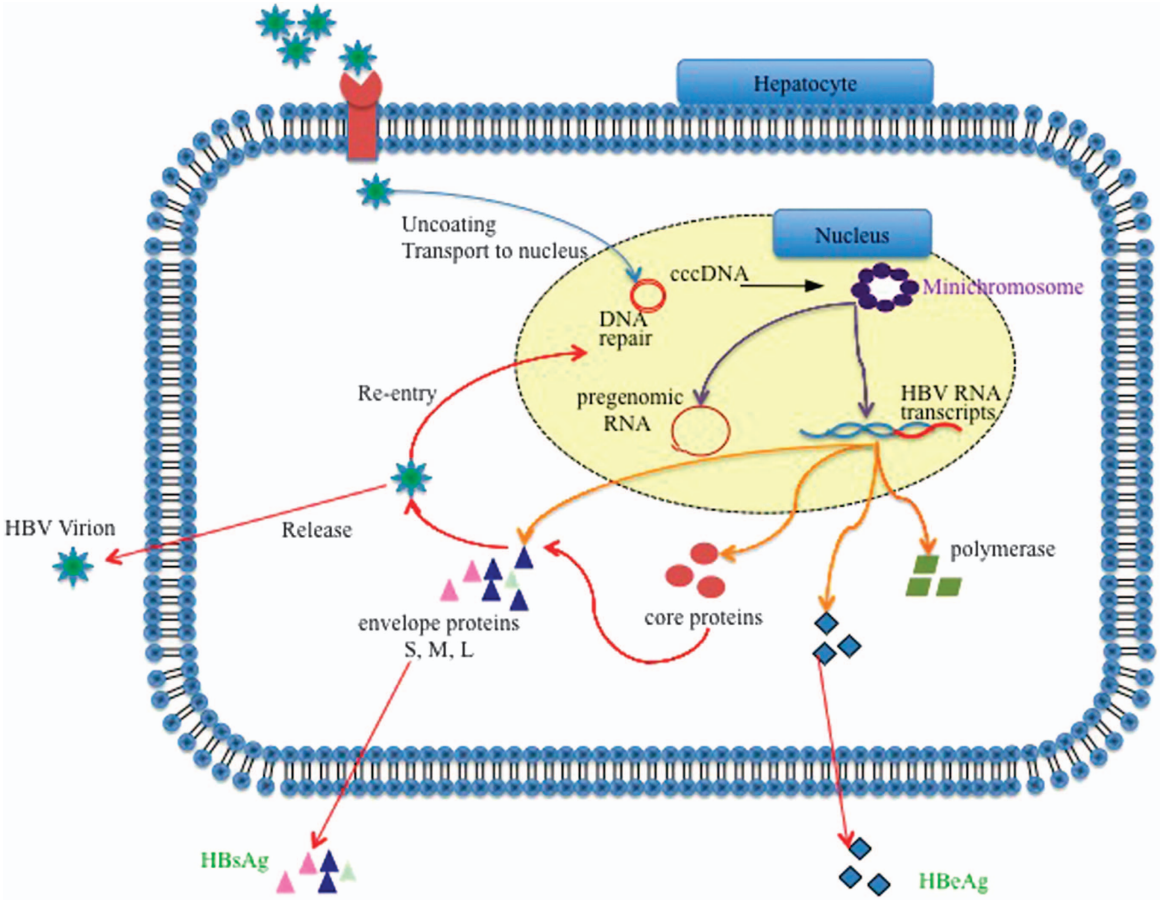

Figure 1 The HBV viral life cycle. HBV binds to the surface of the hepatocyte via the sodium taurocholate cotransporting polypeptide receptor. Surface proteins are removed and the genome is delivered to the nucleus where viral DNA is repaired to form cccDNA and a mini chromosome. HBV proteins are transcribed: core and precore antigen, envelope proteins, X protein and polymerase. HBsAg and HbeAg are secreted. New virions are packaged and secreted, and genomic RNA is recycled back into the nucleus for ongoing viral replication.

therapies, and their widespread use, quickly taught us that TNF deficiency can cause severe complications in the presence of infection due to impaired immunity. The conundrum then is how much TNF is just the right amount and how can we harness endogenous levels to promote clearance of infections agent without causing collateral damage? The purpose of this review is to provide an understanding of the role of TNF in the pathogenesis of HBV with a focus on potential therapeutic strategies.

THE HBV LIFE CYCLE

HBV is a prototype member of the *Hepadnaviridae* family of viruses. It a small virus, 42 nm in diameter made up of a core of partially double-stranded DNA enveloped by a glycolipid shell. HBV binds to the surface of the hepatocyte via the sodium taurocholate cotransporting polypeptide receptor.[10,11] As shown in Figure 1, after entry into the hepatocyte, surface proteins are removed and the nucleocapsid migrates to the nucleus to deliver the genome.[12]

In the hepatocyte nucleus, viral DNA is extended using a reverse transcriptase to form a full double-stranded cccDNA, the functional equivalent of an intracellular provirus. cccDNA binds to histones to form a mini chromosome that serves as the template for transcription of messenger RNA from which HBV viral proteins are transcribed including core antigen (HBcAg), precore antigen (HBeAg), polymerase, X protein (HBx) and surface proteins (HBsAg). HBcAg is not secreted in the serum but stays sequestered in the liver, possibly as a means of viral evasion form the immune system. HBeAg, on the other hand, is secreted and it is thought to be a tolerogen allowing immune evasion and viral persistence. HBsAg is also secreted, in 10 000-fold excess of virions and is abundant in the serum of infected individuals.[13]

Unlike other hepatotropic viruses, HBV DNA can integrate into the host genome. A common site of viral integration is the human telomerase reverse transcriptase (hTERT). This is not necessary for replication but it ensures persistence in the hepatocyte. Integration also confers oncogenic potential to the virus as it can interfere with cellular signaling, proliferation and apoptosis.

Once infected, patients will harbor cccDNA and replication intermediates such as pregenomic RNA in the hepatocyte indefinitely until the hepatocyte is eliminated.[14] Even after seroclearance of HBV, with the formation of antibodies to the surface antigen (anti-HBs), a risk of reactivation is still present in the context of immunosuppression.[14–16]

CHRONIC HEPATITIS B AS AN IMMUNE-MEDIATED DISEASE

HBV itself is not directly cytotoxic and most of the liver injury is mediated by the host immune response. Transgenic mice, which are genetically engineered to have HBV integrated within their genome, are tolerant to the virus and they do not develop liver injury despite high levels of viral replication.[17] Similarly in chimpanzees, at the peak of viral replication, T-cell activity was found to be minimal with no evidence of cytotoxicity.[18] In humans the immune response

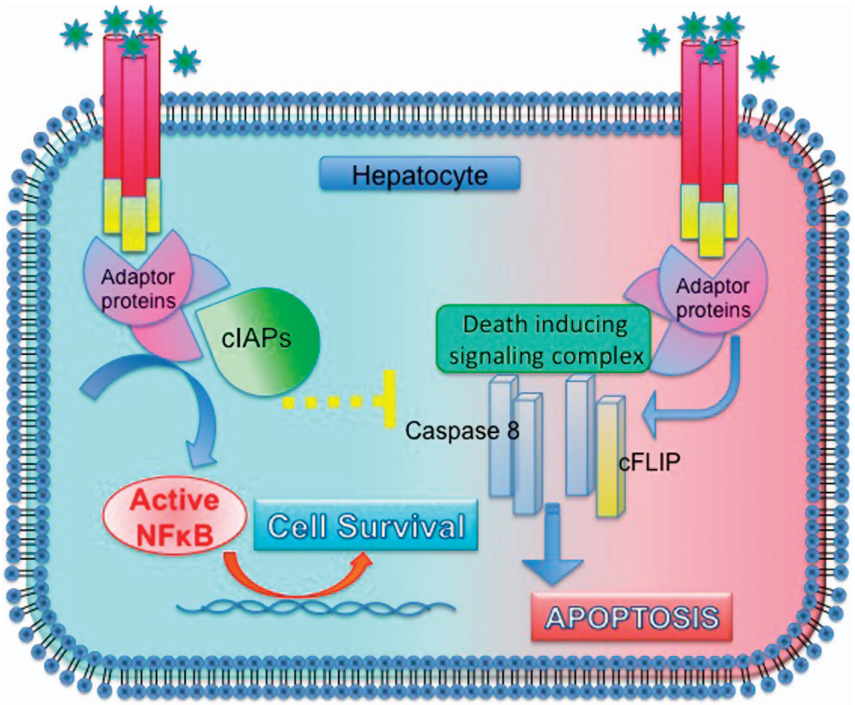

Figure 2 Inflammatory and apoptotic pathways. After TNF binds to its receptor, adapter proteins are recruited and form a signal complex. Recruitment of cellular inhibitor of apoptosis (cIAPs) induces the release of transcription factors like NFkB which upregulates cell survival proteins. However, in the absence of cIAPs recruitment, apoptosis, mediated by Caspase-8, or necroptosis, mediated by MLKL, are the default sequelae.

determines the natural history of CHB and the disease can be separated into distinct clinical phases.[19]

Phases of disease in CHB

In the typical clinical scenario of vertical transmission, it is customary to categorize the natural history into four phases.[20] Phase 1 is characterized by high levels of viral DNA but minimal liver injury and low serum markers of inflammation. This long period of low immunoreactivity is clinically asymptomatic and usually observed in children who acquire HBV perinatally. This phase may facilitate the establishment of chronicity.

In contrast, adults who are acutely infected with HBV will often mount a strong immune response to the virus at the time of infection and have an acute hepatitis. Clinically, this manifests as jaundice, severe fatigue, abdominal pain and flu-like symptoms. Serum amino-transferases are markedly deranged and very occasionally, fulminant liver failure may occur. However, the disease is usually self-limiting and results in virological control with clearance of serum HBV DNA, the formation of antibodies to the virus and lifelong immunity against re-infection from HBV. It was long thought that newborns have a deficient adaptive immune response and therefore naturally tolerate HBV. However, this view has been challenged as neonates have been shown to possess adequate immune maturation with the development of a T helper cell type 1 response, which suggests that the immune response is skewed rather than blunted.[21]

The second phase is that of immune clearance, during which the immune system begins to target HBV-infected cells. Patients may be symptomatic with a clinical syndrome of hepatitis. Biochemically, it coincides with an elevation in liver enzymes (transaminases), a reduction in viral loads, and a drop in surface antigen (HBsAg) titers. Clearance of HBeAg along with the appearance of anti-HBe antibodies (HBeAg seroconversion) often ensues. The natural history of disease continues with flares in viraemia followed by immune-mediated control that falls short of complete HBV clearance. These recurrent flares lead to inflammation and liver scarring. Repeated flares cause a cumulative deposition of scar tissue that eventually culminate in extensive liver fibrosis and cirrhosis.

The third phase of disease is that of immune control whereby the immune system can suppress viral replication. These patients are often referred to as 'silent carriers' of HBV as there are no clinical or biochemical manifestations of disease and the viral load is very low. During this period of viral latency, HBV replication is controlled by the adaptive immune system in particular HBV-specific $CD4^+$ helper T cells, HBV-specific $CD8^+$ cytotoxic T cells, B cells and cytokines such as interferon gamma (IFN-γ) and TNF.[22]

This state can persist for years but the virus can later escape immune control and start replicating vigorously again. Viral replication triggers an immune response and an active hepatitis ensues. Flares of disease can lead to liver failure and death. Immune escape is often due to either the formation of mutants that escape immune surveillance or immunosuppression of the host.[23]

IMMUNOPATHOGENESIS OF HBV

The immunology of HBV is complex, involving interplay of various mediators. The mechanism of clearance is likely a combination of $CD8^+$ T cells that kill HBV-infected hepatocytes, $CD4^+$ T cells and innate immune cells that secrete cytokines which inhibit viral replication, kill infected cells and prevent re-infection. The virus is not passive during this immunological process as it has several characteristics that allow it to evade/escape the host response.[24]

Innate immune system

Following infection with HBV, hepatocytes release Type 1 interferons (IFN-α and IFN-β) that inhibit viral replication.[25–27] Type 1 IFN is

released in response to secreted proteins (HBsAg and HBeAg) as well as intracellular signaling via the JAK-STAT pathway. Cell culture studies have demonstrated that the levels of the replication intermediate, pregenomic RNA, are up to 10-fold less in cells treated with IFN.[26,28] IFN-α/β increases the transcription of interferon inducible genes (ISGs), which inhibit viral replication.[29] IFN-α/β also mediates the activity of antigen presenting cells including Kupffer cells and dendritic cells which then activate natural killer T cells (NKT) through the release of interleukin 18 (IL-18) and CCL3.[30,31]

NK cells are key mediators of innate immunity. They recognize and kill viral infected cells and inhibit viral replication both directly as well as through cytokines in particular IFN-γ or IFN-α/β.[32] NK cells target cells that express little or no MHC class 1 on their surface in the context of upregulated stress ligands and the presence of certain cytokines in particular IFN, IL-12 and IL-18.[33] Hepatocytes generally express low levels of MHC class I and NK cells are abundant in liver making up 30–40% intrahepatic lymphocytes. Therefore, NK cell activation can occur readily with HBV, as even a modest upregulation in stress ligands will permit engagement of NK cells.[34] Transgenic mouse studies have shown that NKT cells inhibit viral replication both directly as well as through cytokines in particular IFN-γ or IFN- α/β.[32]

Although the innate immune system provides initial viral suppression, viral clearance depends largely on the adaptive immune response and a combination of cytolytic killing of infected cells as well as an antiviral cytokine response that promotes death of infected cells, suppresses viraemia and inhibits endogenous re-infection.[35,36]

Adaptive immune response

It is widely acknowledged that the adaptive immune response is responsible for viral clearance in HBV. The humoral arm enables clearance of circulating virus in the serum and limits viral spread whereas the cellular arm eliminates infected hepatocytes. T cells are considered the main effectors of HBV clearance.[37] Animal experiments have provided insights into the role of T cells during HBV infection. Data from chimpanzee experiments have demonstrated an accumulation of $CD8^+$ T cells in the liver during clearance.[18,37] Mouse studies have provided robust evidence that $CD4^+$ T cells are crucial in the immune response to HBV infection, as mice in which $CD4^+$ T cells were antagonized could not control or clear viraemia.[38]

The relative importance of any single immune cell type, whether it be a $C8^+$ T cell, $CD4^+$ T cell or an innate immune cell likely depends on the phase/stage of infection and myriad of host genetic factors, including MHC alleles, and viral genotypes/mutants.[39] However, it is likely that $CD4^+$ T cells have a central role in promoting both $CD8^+$ T-cell dependent and independent killing of infected cells and in facilitating B cell antibody responses.[40]

Antigen presenting cells present peptides to $CD8^+$ and $CD4^+$ T cells which release cytokines IL-12 and TNF that induce the production of IFN-γ and proliferation of $CD8^+$ T cells. IL-12 also induces $CD4^+$ T-cell differentiation into T helper cell type 1 cells. T helper cell type 1 $CD4^+$ T-cells are directed against peptides c50–69, found in both HBcAg and HBeAg.[41,42] IFN-γ is predominantly produced by $CD8^+$ T cells but can also be produced by NKT cells and $CD4^+$ T cells.[31]

Cytotoxic T-cells mediate apoptosis of infected hepatocytes by directly engaging those cells as was demonstrated in transgenic mouse studies using labeled $CD8^+$ T cells.[43] However, direct $CD8^+$ T-cell mediated killing alone is probably insufficient for clearance of HBV.[44,45] Other mechanisms, cytotoxic and non cytotoxic, likely supplement the activity of $CD8^+$ T cells. This concept is supported by transgenic mouse studies which have shown the importance of antibody-mediated viral clearance as well as IFN-γ and TNF-mediated control/clearance.[46] Interestingly, these cytokines have been implicated in destabilizing the nucleocapsid, degrading viral proteins and degrading post transcriptional RNA.[47,48] The sum total of these host defenses is much greater than the contribution of any individual component. Therefore depending on the stage of infection and methods used to investigate the relevance of immunity, certain immunological mediators may appear redundant.[49,50]

TUMOR NECROSIS FACTOR

TNF was first described over 100 years ago and was characterized in the mid 1980's.[51] It earned its name as it was observed to cause disintegration of tumors, and for a long time is was regarded as a group of pro-apoptotic cytokines. The first member of the TNF family to be identified was initially known as cachectin. Other proteins of the TNF family include lymphotoxin, CD40 ligand, Fas ligand, GITR ligand, OX40 ligand, RANK and TNF related apoptosis inducing ligand among others. For the purposes of this review we will exclusively focus on TNF.

One of the remarkable properties of TNF is its pleiotropic quality. As well as being an important mediator of apoptosis, TNF is a major sentinel cytokines whose main biological role is to defend against local injury. TNF has a central role in inflammation and immune regulation as well as cellular differentiation and proliferation. Like most cytokines, TNF is not detectable in quiescent, healthy cells but tissue injury and foreign bodies such as bacteria, parasites and viruses, immune complexes and cancer rapidly induce it. Absence or inhibition of TNF manifests clinically as a failure to control infection.

TNF stimulates the recruitment of neutrophils and monocytes at sites of infection and it activates those cells to enable pathogen clearance. It also acts as a pyrogen on the hypothalamus and increases the production of prostaglandins, which induce fever. In conjunction with IL-1 and IL6, TNF induces the production of acute phase reactant proteins such as serum amyloid protein and fibrinogen from the liver. At low concentration, these pro-inflammatory effects will achieve successful eradication of pathogens with minimal collateral damage. However, when the production of TNF is dysregulated, the outcomes can be deleterious.[52]

At high concentrations, TNF acts as a hormone and has systemic effects that can have fatal consequences. One of the most well recognized destructive effects of TNF is septic shock, which is observed in severe infections in particular with Gram negative bacteria, due to the heavy load of lipopolysaccharides in the bacterial wall.[53,54] Excessive TNF reduces smooth muscle tone and cardiac contractility, leading to a fall in blood pressure, causing cardiac shock. It also disrupts the endothelium and coagulation cascade contributing to the syndrome of disseminated intravascular coagulation and thrombosis.[55] In fact, the mechanisms for the necrosis of tumors that earned TNF its name is via this mechanism of thrombosis of blood vessels that supply tumors. A prolonged exposure to TNF eventually causes cachexia, wasting of muscle and fat cells as it reduces appetite and reduces lipoprotein lipase, the enzyme required to break down lipoproteins for tissue absorption.

Disturbances in TNF are also implicated in the pathogenesis of various autoimmune conditions including multiple sclerosis, rheumatoid arthritis and inflammatory bowel diseases, where it causes inappropriate inflammatory reactions against host tissues. In fact, one of the revolutions in the treatment of those autoimmune conditions has been the use of TNF inhibitors.[56,57] But paradoxically the use of these agents illustrated TNF's critical role in preventing infectious diseases related morbidity and mortality. Antagonizing TNF can

cause reactivation of latent or quiescent infections like tuberculosis and HBV.[58]

TNF is released by activated monocytes, T cells, NK cells, mast cells, B cells and Kupffer cells in the liver. Its production is augmented by IFN-γ, which is released by NK cells and T cells. TNF biology is well described in the literature.[56,59]

TNF-signaling pathways are complex and have been likened to a 'spider's web'.[60] Understanding the pathways for TNF signaling is key to the development of therapeutic interventions that can either inhibit a deleterious over-active immune system as is the case for autoimmune conditions or augment a flailing apoptotic pathway as is the case in CHB.

Just as there is a family of TNF proteins, there is also a family of TNF receptors. TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) are present on most cell types but differ in their expression, their binding affinity, cytoplasmic tail structure and downstream signaling mechanisms. Secreted TNF binds preferentially to TNFR1 and TNFR1 appears to be more important in the function of TNF as TNFR1 knockout mice better recapitulate the phenotype of TNF deficient mice compared with TNFR2 knockout mice.[61] TNFR1 is constitutively expressed on virtually all cell types except erythrocytes, whereas TNFR2 is generally inducible and is preferentially expressed on endothelial and hematopoietic cells.

One of the remarkable characteristics of TNF is that it can either induce gene expression by activating transcription factors and therefore increase the production of inflammatory mediators and survival proteins, or it can induce cell death depending on the metabolic state of the cell.[60] When TNF binds to TNFR1 on the surface of a cell, numerous adapter proteins are recruited to form a signal complex. As shown in Figure 2, the constituents of this signaling complex critically determine the outcome of ligand/receptor interaction. If cellular inhibitor of apoptosis (cIAPs) proteins are recruited to the complex then the transcription factor NFkB is activated resulting in production of cytokines and upregulation of cell survival proteins. However, in the absence of cIAPs the default outcome is programmed cell death caused by apoptosis, mediated by Caspase-8, or necroptosis, mediated by MLKL. The TNF-signaling pathway both pro-inflammatory/cell survival and cell death are reviewed elsewhere.[60]

THE ROLE OF TNF IN HBV

TNF is crucial to viral clearance. A low level of TNF is associated with a weak T cell response and subsequently results in failure to clear HBV.[62] However, it is highly likely that TNF alone cannot clear HBV but works in conjunction with other mediators. In the tree shrew model, TNF could successfully suppress HBV replication but there was persistence of cccDNA.[63]

There are multiple pathways by which TNF exerts antiviral activity. First, as demonstrated in cell culture, TNF decreases viral entry into the hepatocyte.[64] It also reduces viral replication and disrupts the nucleocapsid by interfering with cccDNA through activation of APO-BEC3 deaminases leading to the eventual decay of cccDNA.[47,65] HBcAg has been implicated in promoting TNF-mediated signaling and its downstream effects.[66] TNF signaling can modulate the levels of cellular FADD-like IL-1β-converting enzyme-inhibitory protein (c-FLIP). *In vitro* studies suggest that TNF not only regulates c-FLIP levels but also regulates its conversion to p22-FLIP. Accumulation of the latter protein is thought to inhibit HBV transcription and replication.[67]

Further insights have been provided from animal studies. As previously mentioned, viral clearance, in the chimpanzee model was immediately preceded by a rise in TNF and IFN-γ.[18] Similarly, in a mouse model of HBV infection, peaks in TNF as well as IFN-γ, IL-6, IL-12 and IL-1β were seen in early phases of infection.[38] Yang *et al* also examined the clearance of intrahepatic HBV in a series of immunodeficient mice hydrodynamically injected with HBV and reported that in TNFR1 knockout mice, there was viral persistence.[40] Studies that have antagonized TNF signaling during infection have shown that this treatment causes impairment in virological control,[38,68] a reduction in Toll-like receptor 9 activation and T-cell exhaustion.[69]

The cIAP proteins are critical decision makers in determining whether TNF promotes cellular activation and survival signaling or cell death. As discussed above, in the absence of cIAPs TNF-signaling causes cell death. One study examined if endogenous TNF could be harnessed to promote the death of infected hepatocytes rather than just promoting cellular activation. HBV infection was induced in cIAP deficient mice and these animals rapidly cleared infection with minimal collateral damage.[38] In another study a clinical stage cIAP antagonist was shown to promote clearance of HBV in a preclinical model.[70]

Collectively, these data are corroborated by clinical observations. TNF levels are increased in the serum in patients with HBV[71] and in patients with acute, self-limiting hepatitis; levels of TNF are significantly higher than in patients who harbor chronic HBV.[47,65] Levels of APO-BEC3 are also higher in liver biopsy samples of patients with self-limiting HBV versus chronically infected patients. There is also some anecdotal evidence that shows that the levels of TNF are elevated in patients who clear virus when treated with interferon compared with patients who fail treatment.[72] Genetic studies further support the central role of TNF in HBV. Polymorphisms in the TNF gene can influence the susceptibility to chronicity and outcome of HBV infection such as cirrhosis or HCC. Most of these polymorphisms occur in the promoter region of the gene and of particular interest are nucleotide polymorphisms at nucleotide 308. The 308G allele is associated with viral persistence and 308A has been associated with clearance. The 308A allele was shown to increase TNF-alpha transcription and production.[73] However, other studies suggest an association of the 308A allele with an increased risk of chronic infection.[74] TNF polymorphisms not only appear to be associated with the risk of persistence but may also dictate clinical outcomes including the risks of HCC or liver cirrhosis.[74–76]

USE OF TNF INHIBITORS IN HBV

The use of TNF antagonists including infliximab, adalimumab and etanercept has revolutionized the treatment of inflammatory bowel disease, rheumatological and dermatological conditions. This has been associated with increasing reports of liver damage in patients with HBV. Individuals with inactive or serologically resolved HBV are at risk of reactivation when they are treated with immunosuppressive agents as the balance between immune control and viral replication is disrupted. The risk of reactivation with TNF inhibitors ranges between 1 and 10%.[77] HBsAg positive patients are at significantly higher risk, and one retrospective study quotes a reactivation rate as high as 39% as well as a risk of fulminant liver failure in five patients, four of whom died. However, that data is difficult to interpret due to the concurrent use of other immunosuppressive agents.[78] The risk of HBV reactivation and severe sequalae was significantly reduced with the administration of prophylactic antiviral agents.[78]

In patients who have seroconverted and are HBsAg negative, the risk of reactivation is minimal but the titer of anti-HBs can drop and this warrants surveillance.[79,80] In inactive carriers reactivation manifests as a sudden rise in HBV DNA and serum aminotransferases whereas in those with resolved HBV, HBsAg can reappear.[81,82] When

anti-TNF therapy results in HBV related hepatitis the disease can range from mild to severe and fulminant inflammation. Screening for HBV is therefore recommended before the commencement of immunosuppressive therapies and antiviral therapy may have to be initiated before the start of immunosuppressive therapy.

The type of TNF inhibitor is important as reactivation occurs mostly with Infliximab but not etanercept. Pharmacologically this could be due to the fact that Infliximab binds both membrane-bound and soluble TNF and completely depletes macrophages. The Food and Drug Administration has issued a warning regarding the use of Infliximab in people infected with HBV.

TNF AS A THERAPEUTIC TARGET

Given its central role in HBV clearance, it is an attractive proposition to use TNF to clear chronically infected HBV cells. HBV is virtually incurable once chronic infection has been established, and the available antiviral treatment cannot eradicate cccDNA and does not eliminate the risk of liver cancer due to viral integration. Finding a cure for HBV has significant public implications worldwide.

As detailed above, cIAPs impair the clearance of HBV because they promote TNF-mediated cell survival signaling and antagonize TNF-mediated cell death signaling. Inhibitors of cIAP therefore can augment TNF-mediated apoptosis of virally infected cells and this was shown in an immunocompetent mouse model.[70] It remains to be seen if this strategy can promote clinical cures.

PERSPECTIVES AND FUTURE DIRECTIONS

HBV is an immune-mediated disease, which is a challenge to treat due to a complex pathogenesis. As a result, there is currently no definitive cure that would bring about the permanent clearance of the virus from the liver. Chronicity and persistence of the virus is determined by multiple virus and host factors but basic and clinical research so far strongly supports the association of a weaker or aberrant immune response with chronic, incurable HBV. The need for a cure is urgent in HBV. Currently, all we have at our disposal are interferon and oral antiviral agents. Pegylated interferon is toxic and poorly tolerated and antivirals suppress viral replication but they need to be taken lifelong because they cannot clear cccDNA. Antivirals also do not completely protect against the oncogenic potential of HBV and liver cancer remains a significant cause of morbidity and mortality in HBV patients.

The use of immune modulation is a relatively new concept in HBV. It is a new paradigm of treatment that shifts the focus away from the virus itself and targets the immune response that defines disease pathogenesis. The interferon pathway is an important mediator to the disease pathogenesis. However, by the time most patients are seen in the clinical setting, the contribution of the innate immune system is minimal. The TNF pathway, however, is of great clinical relevance as most patients whom we see in the clinic have had HBV for a number of years and the virus is under the control of the adaptive pathway, of which TNF is the key mediator. We have already seen that manipulating TNF through the use of TNF inhibitors clinically causes a reactivation of virus. Therefore, augmentation of the TNF effect would likely result in viral clearance.

Research in the immunopathogenesis and immune manipulation in HBV has long been hindered by lack of a suitable animal model. Cell culture work has been extremely useful but the analysis of immune pathways is artificial and limited. Many animals have been used including ducks, woodchucks, squirrels and so on but they are not permissive to human HBV. In fact, only the tree shrew and the chimpanzee allow human HBV to replicate. Chimpanzee models, although useful, present major ethical issues. The immunocompetent mouse model that uses hydrodynamic tail vein injection is a solid model in HBV. A potential criticism of studying clearance in that model is that the mouse lacks the sodium taurocholate cotransporting polypeptide receptor and therefore clearance may be somewhat easier to elicit due to the lack of re-infection. However, this replicates the clinical scenario of a patient who is virally suppressed on antivirals.

The use of a clinical stage cIAP antagonist in the model was associated with viral clearance, therefore making it an attractive option for clinical trials.

In conclusion, TNF is a key mediator to immune clearance in chronic HBV. Its interactions with the virus are complex and are yet to be fully elucidated but promoting its function could result in permanent viral clearance and provide a definitive cure for a disease that currently claims over 1 million lives every year.

CONFLICT OF INTEREST

The Walter and Eliza Hall Institute of Medical Research have a research license agreement with TetraLogic Pharmaceuticals (Malvern, PA, USA), the manufactures of the cIAP antagonist birinapant. Tetralogic has filed a PCT on behalf of The Walter and Eliza Hall Institute of Medical Research. MP provides consultative advice to, and was on the scientific advisory board of, TetraLogic Pharmaceuticals. The remaining authors declare no conflict of interest.

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