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RESEARCH ARTICLE

Acute versus Chronic HBV infection. Where do they differ?

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Abstract

Hepatitis B viral infection may occur at birth, childhood or during adulthood. But the number of infected person's resolving infection depends on age at infection. The differentiating factor could be person's immune status. Even though immunization against Hepatitis B virus is available, still owing to absence of flu like symptoms and delayed type of Hepatitis B virus replication, it becomes difficult to diagnose and treat at an early stage. Chronic infection of hepatitis B virus causes liver function deficit, cirrhosis and hepatocellular carcinoma. The role of innate immunity in limiting hepatitis B viral infection in early stages is unclear. In this article we demarcate differences between acute and chronic hepatitis B virus infection and the factors behind each outcome. The main subjects covered are the differences in the microenvironment, immune mediators in liver during acute and chronic infection, strategies used by hepatitis B virus to escape immune response.

Introduction

Hepatitis B virus (HBV) has an unmatched reputation as the most dangerous pathogen infecting human liver and a cause for global concern(Hollinger FB, 2001). Chronic hepatic functional deficit, cirrhosis, and hepatocellular carcinoma (HCC) are the grave consequences of chronic HBV infection. Nearly one fifth of the chronic HBV cases progress to cirrhosis, increasing the risk of developing HCC by 100 folds (El-Serag, 2012). Of all the HCC cases worldwide, every eight in ten are associated with HBV(El-Serag, 2012). It transmits through infected blood, body fluids, and by sexual intercourse via percutaneous or parenteral routes (Aljarbou, 2013) and shows liver tropism. Two modes of transmissions are seen, viz. vertical transmission and horizontal transmission (Li et al., 2015). During first time infection, HBV is confronted by the body's innate defense system (Busca and Kumar, 2014b). The magnitude of immediate innate immune response to some extent is the determining factor that decides the resolution or persistence of HBV infection. This is because time and quality of adaptive immunity are linked to a successful innate response, which in turn depends on factors such as person's immune status, age at infection, inoculum dose etc. If the inoculum dose is large enough, it changes the dynamics of viral spread and innate response (Asabe et al., 2009). In addition, there is a greater chance of CD4+ T cells being primed. It is reported that timely priming of the CD4+ T response prior or during the spread of virus is Indispensable for producing functionally efficient CD8+ T-cell for synchronous clearing of infected hepatocytes. However, this is possible only when the kinetics of viral spread are balanced by the immunologic response. In contrast, HBV persists if CD4+ T-cell are primed later than when HBV infects all hepatocytes (Asabe et al., 2009). Besides maturity of the immune system is also essential for the early immune response, this is the reason why HBV infection is acute in about 90- 95% of infected adults. On the contrary, there is a

risk of about 90% of HBV persistence in infants, owing to their immature immune system (WHO, 2015). There are major differences in the immune responses observed in acute versus chronic HBV infections.

Innate immune response

The main components of innate immune response (Figure. 1) include: phagocytic cells; professional innate immune cells (Figure. 1), that produce cytokines, chemokines, which have antiviral activity (TNF-α, IFN-α and IFN-ß,) or that chemo-attract and activate adaptive immune cells (IL-2,6 & 10). Besides nonprofessional liver cells (HSCs, LSECs and hepatocytes) (Figure. 1) likewise contribute to immune-regulatory functions in response to infection, by secreting cytokines or chemokines (Crispe, 2009).

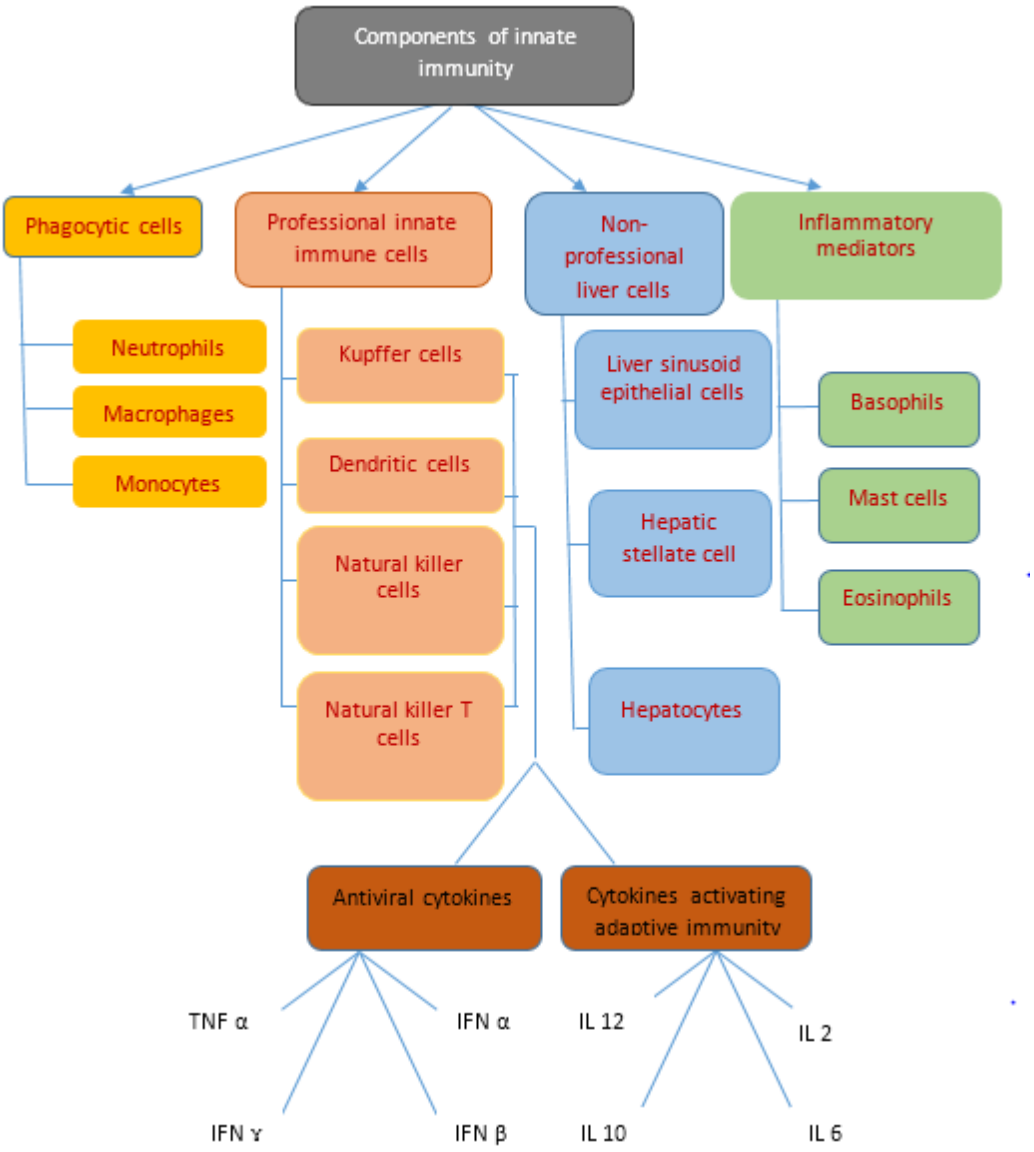


Figure. 1 Components of innate immunity

The first step in fighting the infection is to identify the intruder. The innate immune system makes use of pattern recognition receptors (PRR) to senses pathogen associated molecular patterns (PAMPs). The most crucial PRRs in intracellular viral sensing are, Toll-like receptors (TLRs), RNA helicases (MDA5, RIG-I), and double stranded RNA-dependent protein kinase (PKR) (Thompson and Locarnini, 2007) or Nod-like receptors [NLRs] (Akira et al., 2006). Other receptors such as mannose receptor, scavenger receptor and complement receptors also recognize

PAMPs. It is not clear as to which component of HBV is recognized and which PRR is used in their recognition. However, speculations from some experimental results can be drawn as to which component of HBV is recognized by innate immune system. It is also not known how HBV crosses the liver sinusoidal epithelial cell (LSEC) lining and kupffer cells that separate hepatocytes from blood. It may squeeze through LSEC fenestrae or reach the space of Disse using trans-cytosis through sinusoid cells (Akira et al., 2006). As for the entry of HBV into hepatocyte it has been recently reported that it uses sodium taurocholate co-transporting polypeptide for its entry inside the cell (Yan et al., 2012). However, it may also use other putative transporters as well. Only hepatocytes support the complete life cycle of HBV. There are contradictory views regarding the stealthy or immune-modulatory nature of HBV. In fact, HBV is both stealthy and immune-modulatory that will be discussed in the later section of this review.

During its replication in cell, it produces several viral proteins and components that can be detected by intracellular PRRs (Mogensen, 2009). Newly generated viral particles bud out from hepatocytes and are released in the immediate vicinity. Other components that are released are HBsAg and HBeAg that eventually enter into blood circulation and may interact with components of adaptive immunity. All of these viral components may act as potential PARPs for TLRs of parenchymal and non-parenchymal cells.

Characteristics of HBV infection include delayed HBV replication and spread which often go unnoticed, due to absence of flu like symptoms seen following HBV infections. This lag phase, with no evident viremia, makes it hard to study the role of innate response during human HBV infection. However, suppressive effect of innate immunity may be responsible for delayed replication or that HBV may at first infect a small number of hepatocytes and then spread sluggishly all over the liver (Webster et al., 2000; Wieland et al., 2004). The role of innate immunity in containing initial HBV infection and spread is not clear. Some researchers argue that HBV establishes infection because of its invisibility to immune system and others argue that HBV is immune suppressive and abrogates innate immunity.

Arguments in favour of stealth nature of HBV are, first HBV DNA is recruited and retained in the nucleus for transcription. Second HBV mRNA resemble the normal cellular transcripts which are capped and poly-adenylated and third, HBV pre-genomic RNA is sequestered within viral capsids where it undergoes replication and forms semi duplex circular genomic DNA (Wieland and Chisari, 2005). The hypothesis that, HBV's lack of ability to stimulate type I interferon production and its target gene expression by infected hepatocytes, is backed by these observations. On the other hand, evidences in favour of theories supporting the role played by innate immunity in control of early HBV infection cannot be ignored. Interferon induction may occur in a small number of cells at a given period and the expression of IFN sensitive genes might be below detection limits of the microarray analysis (Guidotti and Chisari, 2006). Recently persons with IFNαR1 gene polymorphisms were shown to be more prone to CHB (Ait-Goughoulte et al., 2010). Besides it was observed that some HBV patients produced low but measurable levels of systemic cytokines/ chemokines such as TNF-α, IFN-α, , IL-10, IL-15 IL-6 and/or IL-1β within 10 days post viral spread and prior to peak viremia (Zhou et al., 2007). Clues from These findings indicate that there is some interaction of HBV with the components of innate immunity. Further supporting the arguments favouring role of innate immunity in HBV infection are the experimental results by Lucifora et al. that verified a strong HBV replication is a prerequisite for type I IFN response in infected cells(Lucifora et al., 2008). Besides in HBV infection models, innate immune responses are activated only by robust and brisk increases in levels of HBV replication. This implies quantity rather than the 'quality' of HBV DNA triggers intracellular innate response. The concept of size and speed of viral replication are being realized in the virus–host relationship (Unterholzner and Bowie, 2011). In line with this it is also observed that a sudden surge in HBV DNA and antigens levels in blood precedes hepatic flares (Dunn et al., 2007; Tan et al., 2010). This led to the hypothesis that hepatocytes, which express very few MHC class-I molecules, are augmented for antigen presentation and MHC class-I molecule expression in response to intrahepatic pro-inflammatory cytokine release enhanced by HBV viral antigen accumulation. Assuming that innate immunity does play a role in controlling initial HBV infection, we should now explain the differences between acute / acute HBV infection and chronic / chronic HBV infection.

Acute HBV infection

Liver is viewed as an organ with innate immune features, due to abundance of innate immune cells, and is believed to be important in the first-line host defense, countering pathogens (Kanto, 2008). Activation of innate cells is linked to a positive clinical outcome and subsequent strong adaptive immune responses, following acute infection or immunization (Busca and Kumar, 2014a). Pro-inflammatory mediators play a crucial role in promoting effective virus-specific immunity. They inhibit viral replication, induce resistance to infection in neighbouring cells, and recruit and activate other immune cells (Boltjes et al., 2014; Lang et al., 2010; McDonald et al., 2010; Wu et al., 2007a). Still a complete and effective resolution of hepatocellular viral infection requires CD8+ T cells and multispecific adaptive

immune response. Following is the detailed account of how innate immunity paves way for a successful adaptive immunity to take over the process of clearing HBV infection.

Role of macrophages and kupffer cells.

Macrophages are phagocytic cells differentiated from monocytes. Monocytes in circulation migrate into tissues, where the immune cells, chemokine milieu and the extracellular matrix (de Fougerolles et al., 2000; Ma et al., 2003; Monney et al., 2002) determine gene expression changes and trigger their differentiation. Kupffer cell (KC) are long-lived resident macrophages of liver, embedded in between the liver sinusoidal endothelial cells (LSECs). Macrophages and KCs bridge innate and adaptive immune responses (Heydtmann, 2009) with a cascade of innate inflammatory responses as described below.

Macrophages and KCs express TLR1 through TLR6 and TLR8 (Szabo et al., 2007). HBV capsids are recognized by TLR2 on these cells (Dolganiuc et al., 2004). Besides Macrophages and KCs may be activated in response to binding of phagocytized HBV to endosomal TLR8 (Heydtmann, 2009). Kupffer cells and other cells may also get exposed to free viral nucleic acids and proteins that can be detected by membrane bound PRRs in the later stages of HBV infection (Szabo et al., 2007).

Kupffer cells makeup about 15-20% of the total liver mass (Jenne and Kubes, 2013). They are the first type of cells encountering materials absorbed in the gut. Together with the sinusoidal endothelial cells, KC are the first barrier for pathogens entering the liver via the portal vein (Kolios et al., 2006). KCs promote general tolerogenic liver environment, (Kolios et al., 2006) besides suppressing T-cell activation in the liver (Matlack et al., 2006). Nevertheless due to their abundance and localization, KC are crucial in rendering intrahepatic innate immunity. They are specialized to perform scavenger and phagocytic functions, removing protein complexes, small particles, and apoptotic cells from blood (Parker and Picut, 2005; Vollmar and Menger, 2009). During immune responses, KCs can be activated by various stimuli (Doyle et al., 1994). Other than above-mentioned methods, they probably also sense HBsAg in CD-14 dependent fashion and through mannose receptors (Boltjes et al.; Vanlandschoot et al., 2002). Besides, albumin bound HBsAg may be taken up from the circulation by KC and endothelial cells (Wright et al., 1988). C-type lectins are important receptors mediating phagocytosis and are expressed by human KCs (Dominguez-Soto et al., 2009). Also damage-associated molecular pattern (DAMP), may overcome liver intrinsic immune-tolerogenic state and viral immune escape. DAMPs provide a synergistic signal to PAMP-driven immune activation that promotes hepatic inflammation (Canbay et al., 2003). Activated KCs possibly kill hepatocytes by several mechanisms. KCs have been reported to express cytotoxic molecules such as perforin, granzyme B, TRAIL, Fas-ligand, and ROS, enabling them to lyse infected hepatocytes (Tordjmann et al., 1998). Greater number of FasL expressing KCs are detected during episodes of liver damage in HBV infected individuals (Tang et al., 2003). This expression of FasL promotes hepatocyte apoptosis (Jenne and Kubes, 2013). KC-derived TNF damages hepatocytes as shown in many models of T cell mediated acute liver damage (Polakos et al., 2006).

Release of chemokines and cytokines by KC recruit and activate infiltrating leukocytes, as do macrophages. Upon HBV exposure, KCs produce CXCL8, (Figure.2 step 5) (Cooper et al., 2005) which potentially attracts NK and natural killer T (NKT) cells during the early phase of HBV infection. Besides, KCs in response to Type I IFN also produce CCL2 (Figure. 2 step 2) that recruits cells monocytes in blood circulation to the liver, where they release CCL3, which triggers NK cell recruitment (Figure. 2 step 4). NK cells produce high levels of IFN-ɤ. Macrophages in presence of IFN-ɤ, undergo classical adaptive activation, inducing CXCL9 production. CXCL9 along with CCL3 recruit CD4 T cells (Figure. 2 step9).

Macrophage activation results in release of pro-inflammatory cytokines and chemokines. Consequently, acutely infected individuals with HBV, have increased plasma levels of IL-6 and IL-10, which are immune modulatory and protect tissue from immune mediated liver injury (Hosel et al., 2009). Macrophages activated by innate immune components often up regulate expression of major histocompatibility complex class II (MHCII) molecules, thus increasing the priming of CD4 cells that then differentiate in to T helper cells (Kolios et al., 2006). Besides classical adaptive activation of macrophages results in their enhanced cytotoxic effect (Ehrt et al., 2001).

Role of natural killer cells

Increasingly more studies indicates that NK and NKT cells are major determinants of HBV infection. Their activation occurs promptly before peak HBV DNA levels and before HBV-specific T cell response (Fisicaro et al., 2009). Natural killer and NKT cells, both present in large numbers in liver, are activated by pro-inflammatory cytokines-IL12 and IL18 released by KC (Figure2. Step 6) (Biron and Brossay, 2001; Tu et al., 2008). Besides monocytes recruited by KCs produce CCL3, another attractant of NK cells. In turn, NK and NKT cells produce

cytokines such as TNF and IFN γ and are cytotoxic in nature[53] in addition NKT cells also produce IL-4. (Biron et al., 1999; Trobonjaca et al., 2001) IL-8 can also potently chemo-attract NK cells and up-regulate TRAIL death-inducing receptors on hepatocytes. On the other hand TRAIL expression on NK cells is induced in response to increase in IFN-α production. This means NK cells could kill hepatocytes through the TRAIL pathway, (Figure.2 step 13) which is observed in patients undergoing flares.

Evidences suggest that NK and NKT cells are also activated directly by HBV glycolipids, phospholipids and up regulated cellular stress signals on infected hepatic cells (Kakimi et al., 2000). These may engage NK activating receptors and induce local NK cell effector function which is influenced by the local cytokine milieu (Dunn et al., 2007).

Studies have demonstrated that the number of NK cells in circulation increase during the time of first HBsAg detection and HBV replication (Webster et al., 2000). Besides a Longitudinal study showed a temporal correlation between the fluctuating levels of IL-8, IFN-**α** and TRAIL expression on NK cells and the occurrence of flares of liver inflammation (Dunn et al., 2007). IFN-α which promotes cytotoxicity and TRAIL expression (Nguyen et al., 2002; Sato et al., 2001) and IL-12 favouring IFN-γ may also promote NK mediated viral clearance and liver injury (Nguyen et al., 2002).

Activated NK cells kill infected cells either by direct cell-to-cell contact or by releasing inflammatory cytokines (Biron et al., 1999; Guidotti and Chisari, 2001). TNF superfamily ligand-receptor pairing is a likely mechanism for Receptor-mediated liver cell death. One such example is TRAIL expressed on lymphocytes interacting with its receptors (TRAILR1 and TRAILR2) on hepatocytes (Dunn et al., 2007). NK cells produce cytokine such as TNF-α and IFN-ϒ that have antiviral activity and those with immune-modulatory activity, such as IL-3, GM-CSF, and M-CSF (Ratnam and Visvanathan, 2008).

Role of dendritic cells

KCs also recruit dendritic cells to the liver (Schulz et al., 2005; Uwatoku et al., 2001). They are of two main subtypes. Myeloid dendritic cells (mDC), they act as antigen presenting cells and in fact the most potent and only ones capable of stimulating naïve T cells. Besides they can present antigens on both class I class II MHC molecules. Other type is the plasmacytoid dendritic cell (pDC), which secrete significant amounts of type 1 IFNs (Figure. 2 step 12) and play a fundamental role in immune responses countering viral infections (Banchereau and Steinman, 1998; Colonna et al., 2004; Ito et al., 2006)_ DCs take up virus-infected apoptotic cells through the endocytic receptor C type lectin 9A (CLEC9A) (Uwatoku et al., 2001) Besides TLR receptors in their endosomal or lysosomal cell compartments also recognize viral antigen and nucleic acid sequences, triggering functional maturation of DCs (Ito et al., 2006) and help in cross-priming CTLs (Figure. 2 step 12). pDCs also produce IL-6, TNF-α and co stimulatory molecules on the cell surface (Ito et al., 2005). Hepatocytes in response toIL-6, express acute phase C-reactive protein which is an innate effector molecule. Type I IFNs besides having potent antiviral effects, improve antigen presentation (Baumann and Gauldie, 1994). Besides activating KCs, type I IFN also activate platelets that increases CTL-mediated liver damage (Iannacone et al., 2005; Iannacone et al., 2008). Activated CTL produce CXCL-9 and CXCL-10, which are necessary for chemo-attracting inflammatory cells to liver (Tan et al., 2010). Plasmacytoid DCs also activate NK and T cells, thereby further priming and regulating anti-viral immunity (Iannacone et al., 2005; Schulz et al., 2005).

Thus, KCs and macrophages bridge innate and adaptive immune responses by integrating cascades of inflammatory events. Patients with a self-limiting HBV infection present an incessant, strong, and multi-epitope-specific CD4 + or CD8 + T cell and B cell responses, whereas in chronic HBV these responses are weak and/or transient (Boltjes et al., 2014; Day et al., 2002; Lauer et al., 2002; Thimme et al., 2001). This demonstrates that clearance of the infection is dependent on strong multi epitope-specific T and B cell responses, which prior to which effective innate immune responses must occur(Boltjes et al., 2014). Early activation of adaptive immune response limit viral replication and spread, thus reducing initial viremia without significant damage to liver (Guidotti and Chisari, 2001). Nevertheless CTL-mediated cytotoxicity is also required for clearing infected cells (Ferrari et al., 1990; Rehermann et al., 1995), CD4 differentiation into T helper cells is also important in providing humoral immunity from circulating HBV particles.

Chronic or persistent HBV infection

Chronic HBV infection seems to establish in individuals with weak immune system and infants. As mentioned earlier, there is a greater chance for young children becoming chronically infected as compared to adults. It is conceivable because young children are in the developing stages of immunity. Besides their food, composition

differs from adults; which may establish different or not enough gut microbiota, which play an essential role in maturity of the immune system. Liver being the organ supplied directly from portal blood may contain microbial exudates that may also suppress the innate immune system (Compare et al.; Vajro et al., 2013). However, the main factor determining resolution and persistence of HBV infection seems to be a successful innate response and timely activation of multispecific T cell response. It is observed that the quality and quantity of T cell response is downgraded in chronic HBV infection. Of course, factors such as inoculum dose, person's age and immune status also determine the outcome of host – viral interaction.

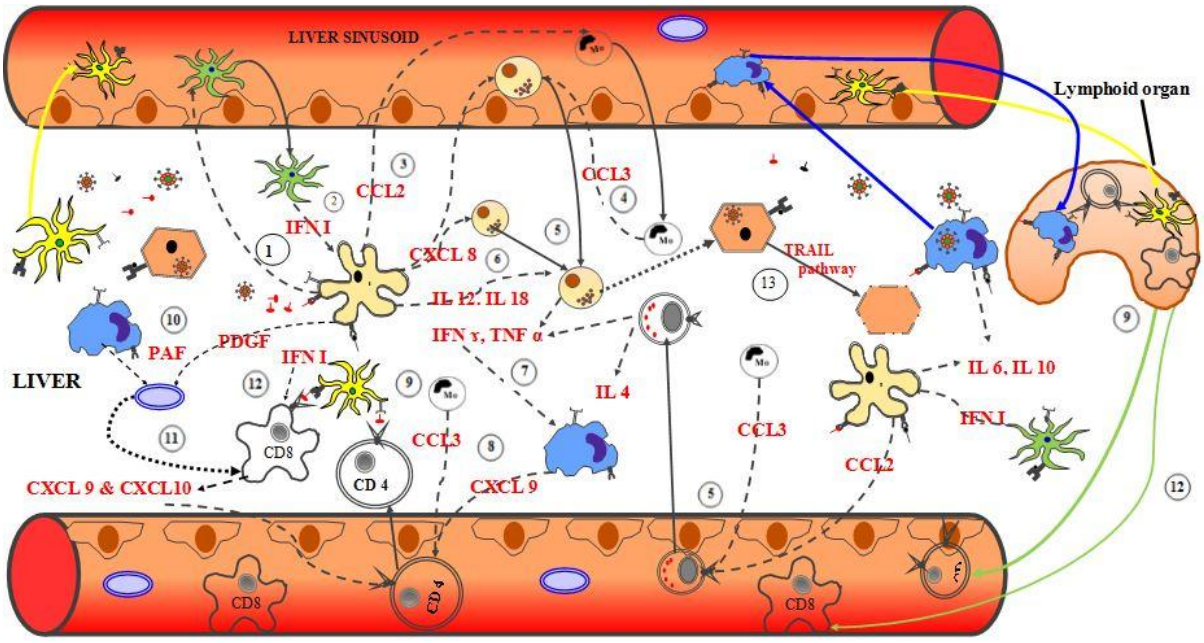
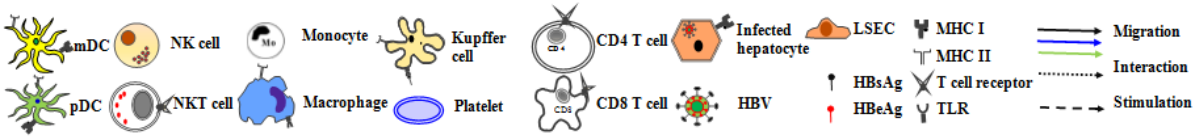


Fig 2. Innate immune response in acute HBV infection. 1. KCs in response to HBV and its proteins release inflammatory mediators that recruit other innate immune cells including DCs to the site of infection. **2.** pDCs release type I IFN (IFNI). KCs in response to IFN I and HBV release CCL2 and CXCL8 respectively. **3.** CCL2 recruits monocytes to the site of inflammation. **4.** Activated monocytes release CCL3. **5.** CCL3 and CXCL8 chemo-attaract NK and NKT cell to the site of infection. **6.** NK and NKT cells are activated by IL12 and IL18 released by activated KCs. Activated NK cells release IFNγ and TNFα.**7.** IFNγ triggers classical adaptive activation of macrophages that up regulate MHCII molecules and release CXCL9. **8.** CXCL9 along with CCL3 released from monocytes recruit CD\$ T cells. **9.** mDCs and other antigen presenting cells and macrophages present antigens on their MHCII molecules in the infected liver as well as in lymphoid organs, where CD4 cells mature in to TH1 cells. **10.** platelet activating factor (PAF) released from macrophages along with platelet derived growth factor released by KCs activate and recruit platelets **11.** platelets recruit CD8 T cells that are activated by IFN I released from pDCs **12.** Along with hepatocytes expressing MHCI, professional antigen presenting cells such as DCs can cross present Ag on their MHCI molecules to CD8 cells. Activated CD 8 cells release CXCL9 and CXCL10 that amplify adaptive immune cell response . NOTE: this diagram is not the actual anatomical depiction of liver tissue.

Legends



Studies conducted on HBV inoculum doses have demonstrated an inverse relation to persistence. Usually chronicity is seen with very low HBV inoculum dose at first infection. As compared to acute HBV infections, the kinetics of HBV infection and spread and that of innate immune response differ. Low HBV doses go undetected and successfully infect few hepatocytes. This could be because of immune-tolerance shown by healthy liver. HBV's behaviour as compared to other viruses is peculiar, as it does not cause antiviral Type I IFN release (Tang et al., 2003). This can be due to its stealth or immune suppressive nature as discussed earlier. Besides just because of sheer small number, innate response elicited within the microenvironment of infected hepatocyte, may not be enough to mount a strong inflammatory response. This may give virus a scope to show its immune-modulatory effect as it spreads from focal point of infection increasing its boundaries, until it completely infects liver. This may cause an ineffective continued stimulation of resident hepatic innate cells and prolonged inflammation. This may be the main reason for persistence of infection. Therefore, it is important to know how HBV causes immune suppression and modulation in the microenvironment of cell it infects and beyond.

Strategies of HBV in innate immune ablation

HBV seems to confront innate immune setting at three levels. First, it abrogates IFN production and its downstream signalling of PRRs inside cell particularly the IFN induction and response. Second, it modulates innate cell function through its proteins. Third, it secretes large amounts of HBsAg and HBeAg, which besides altering innate cell function also engage humoral and cellular immune components, which are overwhelmed by viral proteins.

HBV protein interference with interferon signalling components

Chronic HBV infection may occur due to evasion of innate immune response by HBV through down regulating interferon pathways (Ait-Goughoulte et al., 2010), that are the major innate defence mechanisms against viral infections. HBV and its proteins first inhibit the induction of interferon (Figure. 3) and then modulate downstream effector molecules in IFN response (Figure 4).

It is reported that HBx protein actively interferes with RIG-I, helicases (cytosolic sensory molecules) interaction with the IPS-1 (β IFN promoter stimulator 1), which is essential for the induction of type I IFN (Christen et al., 2007; Kumar et al., 2011; Wang et al., 2010; Wei et al., 2010; Wu et al., 2007b). As a result double-stranded DNA mediated IFN-β release, in hepatoma cell lines is inhibited (Kumar et al., 2011). Besides Polymerase interferes with IRF (IFN regulatory factor) signaling. Mechanistically, polymerase interacts with DDX3, a transcriptional factor of the IFN-β promoter. As a result IFN-β release is inhibited (Christen et al., 2007; Kumar et al., 2011; Wang and Ryu, 2010; Wang et al., 2010; Wei et al., 2010; Yu et al., 2010). Thus HBx and polymerase inhibit the innate immunity.

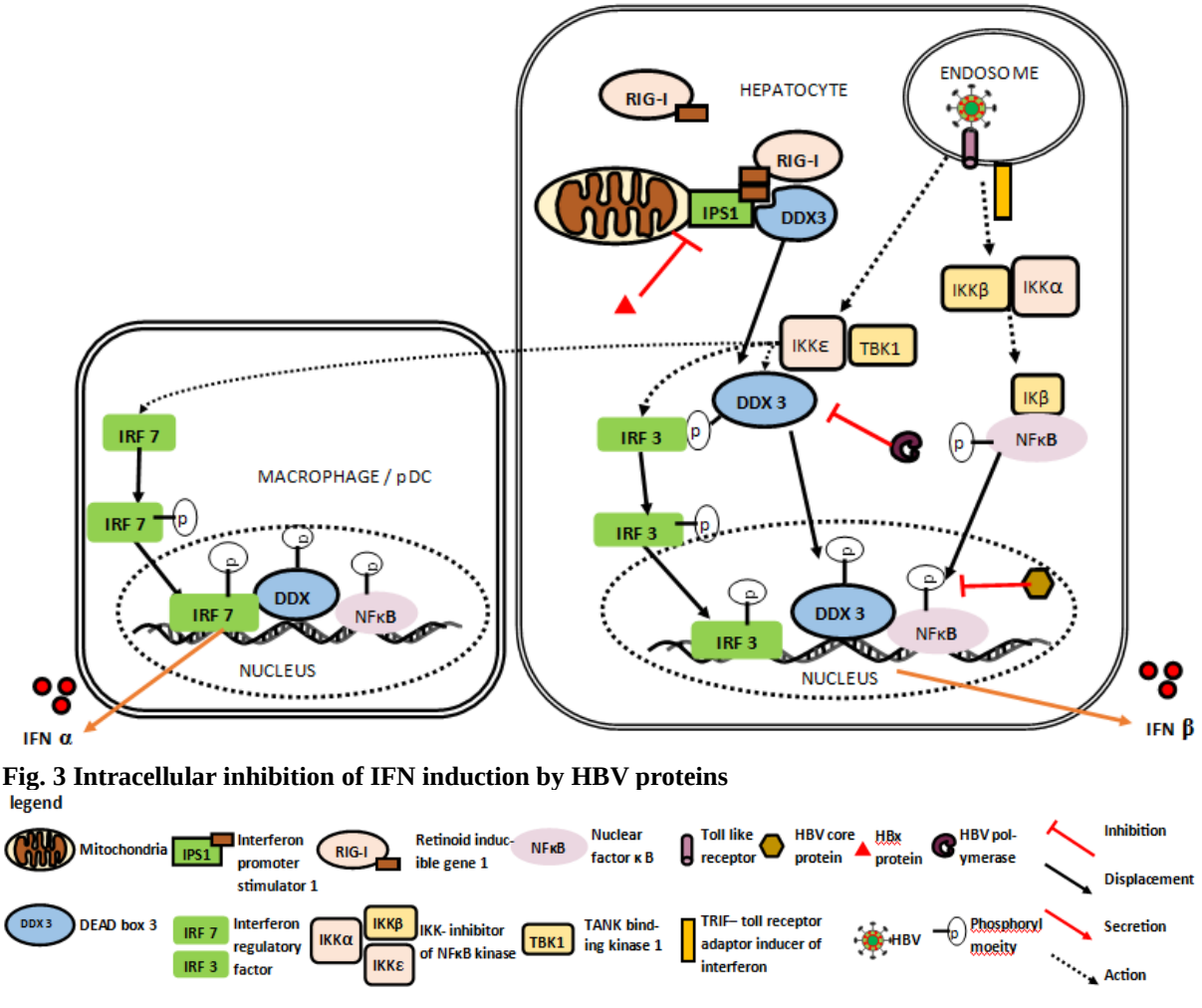


Fig. 3 Intracellular inhibition of IFN induction by HBV proteins

HBV and the terminal domain of HBV polymerase protein interfere with ISG promoter activity by blocking Stat1 nuclear import. Thus inhibiting the transcription of ISGs such as MyD88 (Lutgehetmann et al., 2011). It is also observed that HBV core protein accumulation in cytoplasm, strongly inhibits MxA expression, hence reducing the antiviral activity of IFN-α (Rosmorduc et al., 1999). Additionally, ISRE promoter transcriptional activity, expression of ISGs (ISG15 and STAT1) were also inhibited (Wu et al., 2007b). S and/or X proteins, could also block STAT1 function by up-regulation of pp2AC- a cellular protein that inhibits PRMT1 resulting in reduced STAT1 methylation. Therefore, un-methylated STAT1 binds to PIAS1 and has a decreased capacity to stimulate IFN-target genes (Kumar et al., 2011). These finding suggest that HBV proteins not only block the induction of IFN release by targeting key components in intracellular PRR signal components, but also inhibit different components of the IFN signaling pathway.

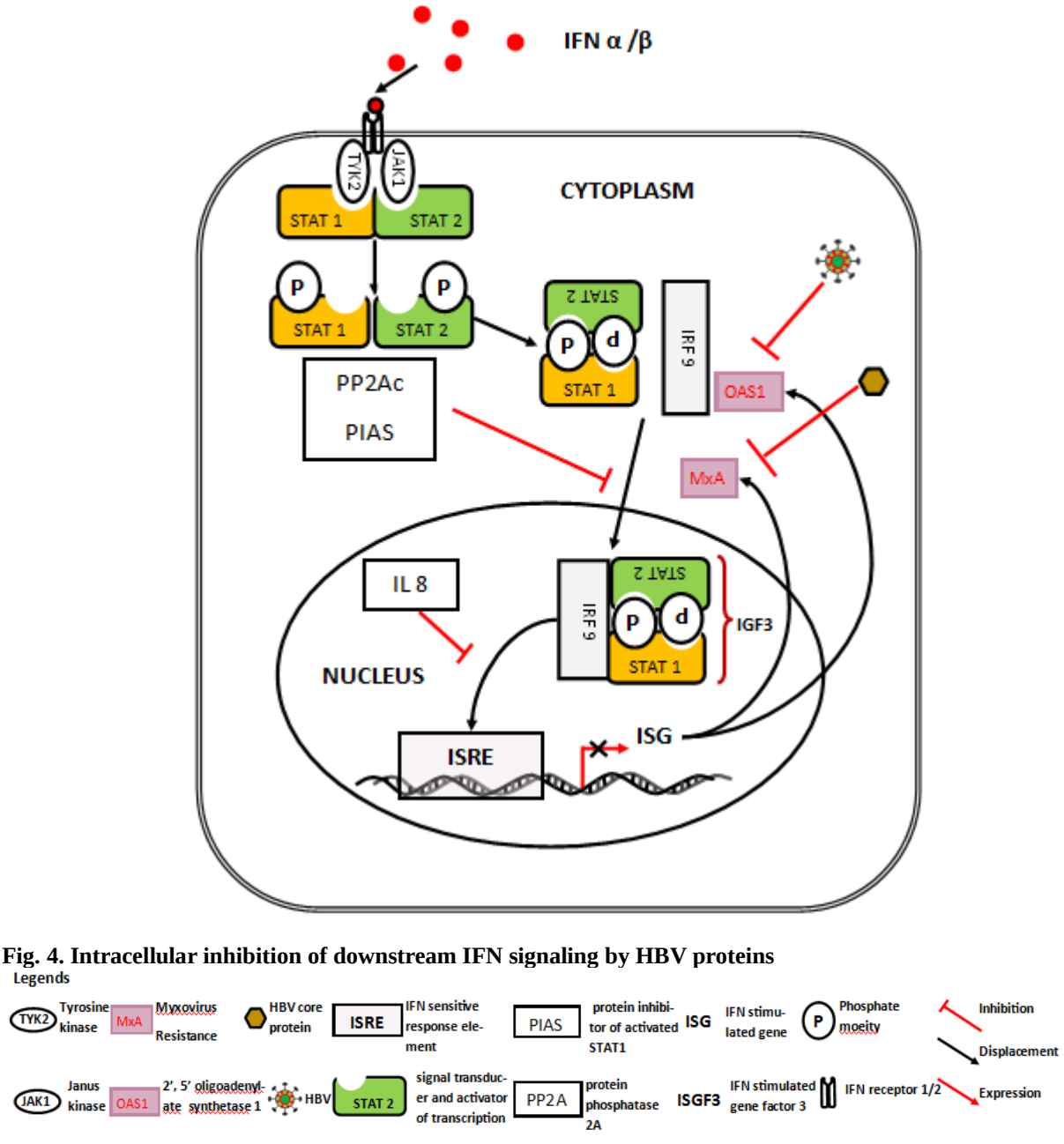


Fig. 4. Intracellular inhibition of downstream IFN signaling by HBV proteins

HBV protein interference with TLRs

HBV suppresses innate immunity not only by inhibiting type I IFN induction, but also by countering the effects of IFN-α or –β produced by DCs, KCs and non-infected parenchymal cells, and KCs, or even exogenously administered interferon (Lutgehetmann et al., 2011; Rosmorduc et al., 1999). Growing number of evidences suggest that HBV could also inhibit innate by regulating the expression of Toll-like receptors (TLRs) (Barton, 2007), and/or inhibit TLR signaling cascades. The production of HBeAg and HBsAg has been associated with interference of adaptive immune mechanisms (Milich and Liang, 2003). Moreover in CHB, patients the production of cytokines by PBMCs was inhibited in response to both TLR-2 and TLR-4 binding and this inhibition was associated with the level of HBsAg (Chen et al., 2008). HBsAg is reported to reduce release of IL-12 and IL-18 BY KCs and also inhibits lipopolysaccharide-induced expression of cyclooxygenase-2. Besides it also interferes with the monocyte function by inhibiting NFκB and ERK pathway (Cheng et al., 2005). Also activated macrophages produce less TNF in vitro when exposed to lipopolysaccharide (TLR4 ligand) in the presence of HBV particles (Biermer et al., 2003). Reduction in monocyte function would decrease NK and NKT cells. While reduction in the expression of IL-12 and IL-18 would reduce the activation of NK and NKT cells. Which would impair NK cell capacity to release TNF and INF ɤ (in response to IL-12) (Tu et al., 2008). As mentioned earlier IFN ɤ induces classically activated macrophages to release CXCL9. CXCL9 in combination with CCl3 released by monocytes recruits CD4 cell. Thus, impairment of monocyte and NK and NKT cell function would interfere in recruitment of CD4 cells and hence prevent virus specific adaptive immunity. A recent report of TLR-9 expression reduction in pDCs of CHB patients, correlates with impaired IFN-α production (Xie et al., 2009). This could affect TRAIL receptor expression on hepatocytes making them less susceptible to TRAIL mediated apoptosis, induced by NK cells. TLR3 down regulation and decreased IFN-ß, its downstream cytokine production in MoDCs of CHB patients was also observed (Li et al., 2009). Downregulation of TLR-2 expression in PBMCs, KCs and hepatocytes from HBeAg positive patients was recently described (Riordan et al., 2006; Visvanathan et al., 2007). Moreover, TLR2 ligation induced less IL-6 and TNF in those HBeAg-positive patients (Li et al., 2009). Binding of HBeAg or HBsAg inhibited TLR2-induced phosphorylation of p38 MAPK and JNK MAPK, and subsequent production of TNF, IL-6, and IL-12 (Riordan et al., 2006; Visvanathan et al., 2007; Wang et al., 2013). These alterations may be related to the inhibitory effect of HBeAg on TLR2 signaling demonstrated in vitro. IL-6 induces innate effector molecules. Altogether, these findings strongly speak for HBV mediated inhibition of TLR functions that could alter the innate immune response mediated by both specialized cells and hepatocytes, which may result in of chronic infections.

Depletion of virus specific CTLs and antibodies

In CHB virus, specific CTLs are found to be depleted or their functions modulated in favour of persistence of viral infection. CTL depletion can be partly attributed to their enhanced apoptotic tendency levied by tolerogenic priming of liver, which through BCL-2-interacting mediator of cell death (BIM) induces T cell death (Bowen et al., 2004; Holz et al., 2008; Lopes et al., 2008; Schurich et al., 2011). BIM-mediated apoptosis is carried out by co-inhibitory signals mediated by cytotoxic T lymphocyte antigen 4 (CTLA4) or by T cell-intrinsic TGFβ (Schurich et al., 2011; Tinoco et al.). Besides another important cause of T cell exhaustion is an excess of co-inhibitory signals that overshadows the co-stimulatory signals. This is best shown by a co-inhibitory molecule viz. programmed cell death protein 1 (PD1). It tightly regulates T cell reactivity to prevent autoimmunity (Dong et al., 2004; Iwai et al., 2003). Also humoral immunity is blocked by large excess of HBsAg secreted by infected hepatocytes in CHB patients. This saturate the circulating antibodies against HBV, preventing virus neutralization.

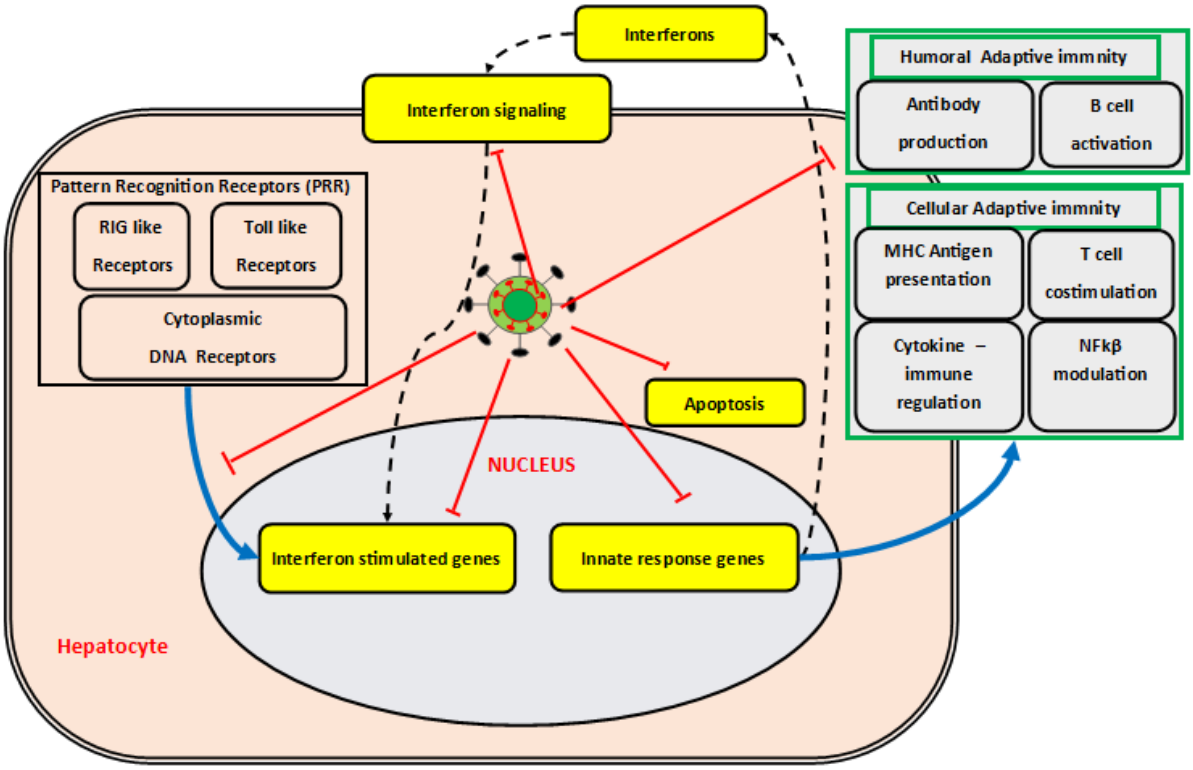


Fig. 5 Levels of immune modulation by HBV

Macrophage and kupffer cell functional deficit

Macrophages and KCs show decreased stimulatory function in CHB. This may be due to the inhibitory milieu in the infected liver microenvironment. The activation of PRRs by HBV stimulates release of IL-10 by Kupffer cells and LSECs (Tang et al., 2003). As mentioned earlier, IL-10 down regulate MHC-II expression on macrophages (Gordon, 2003). Besides they show reduced capacity to produce TNF and IL-12. Moreover, TRegs also inhibit IFN ɤ secretion (Peng et al., 2008) that is needed for classical adaptive activation of macrophages, hence resulting in their decreased cytotoxic activity(Ehrt et al., 2001).

HBV also interfere with the pro-inflammatory functions of KC to evade host immunity. It inhibits expression of cyclooxygenase-2, reduces IL-12 and IL-18 by KC through NFκB and ERK pathway inhibitions (Cheng et al., 2005). HBV also abrogates its anti-viral activity by inhibiting TLR induced IFN b production, ISG induction, IRF3, NFκB, and ERK1/2 expressions (Wu et al., 2009). Besides as mentioned earlier there was down regulation of TLR-2 expression on KCs which induced less IL-6 and TNF in HBeAg-positive patients (Tang et al., 2003), implicating a viral evasion strategy to inhibit this antiviral cytokine. In contrast to DCs, KCs are less effective in priming naïve T cells. A weak ability of KC to produce cytokines might be related to their tolerogenic function in a steady state condition. KCs constitutively express TGF-β and PD-1 and possess high levels of negative regulators downstream the TLR pathway and secrete IL-10 upon LPS stimulation (Iwai et al., 2003; Kinoshita et al., 2010; Knolle et al., 1995; Liu et al., 2008; Movita et al., 2012; Roth et al., 1998).

Down regulated natural killer cell function

The main function of NK and NKT cells is to limit viral replication not by cytotoxicity but rather by cytokine (IFN-γ and TNF-α) production, which is compromised during CHB infection, which may be responsible for HBV persistence (Busca and Kumar, 2014a; Fisicaro et al., 2009). However, NK cells also develop tolerance in the liver, where they fail to produce IFNγ but retain their cytotoxic potential (Oliviero et al., 2009; Peppa et al., 2010). This NK cell tolerance may be mediated by IL-10-producing KCs and LSECs (Fisicaro et al., 2009; Roth et al., 1998). This inhibition of NK cell activation occurs in response to IL-10 (Dunn et al., 2009).

Dendritic cell functional deficit

Dendritic cells (DC) not only generate and coordinate adaptive immune responses but they also induce tolerance to self and foreign antigens. Promoting conversion of naïve T cells to TRegs is one mechanism by which pDCs contribute to immunological tolerance in healthy individuals. Higher frequency of TRegs were observed in CHB patients that correlated with increased DC number and HBV DNA concentration in serum (Manigold and Racanelli, 2007). Treg cells limit the function of effector T cells preventing excessive auto-destructive disease (Jeulin et al., 2013). However, during CHB infection they seem to have a damaging effect, because they increase in number as compared to acute infection. Type I IFN producing pDCs, are the targets of HBV for producing an immune-tolerant niche in the infected liver. The presence of HBV or HBsAg during cytokine-induced maturation of DCs resulted in a more tolerogenic phenotype, as shown by a reduced T cell stimulation (Op den Brouw et al., 2009). Functional deficits of pDC from CHB patients may be due to decreased TLR9 expression levels which has been recently reported (Xie et al., 2009). TLR9 mediates TNFα release which was reported to be inhibited by pDC-specific C-type lectin DC immunogenic receptor (DCIR) crosslinking (Meyer-Wentrup et al., 2008). Besides it was observed that reduction of TLR9 expression also correlated with impaired IFN-α production by pDCs that may impair pDC's ability to induce cytolytic activity of NK cells (Martinet et al., 2012). in addition TLR3 was also down regulated markedly in MoDCs thus reducing a downstream IFN-ß production (Li et al., 2009). Suppression of IFN-β production subsequently decreases the activation of ISGs (e.g., IP-10, MxA, pERK or NFκB) (Wu et al., 2009).

Inhibition of adaptive immunity

Chronic viral infection in the liver is propagated through several mechanisms (Protzer et al., 2012), which may have evolved to protect the liver from immune-mediated damage. In chronically infected patients, a feeble and focused T cell response is observed (Maini and Bertoletti, 2000). This is because, besides exhaustion of CD8+ T (CTL) cells, their function is also altered (Figure 6). CTLs are also sensitive to co-inhibitory molecules that promote tolerance towards HBV infected hepatocytes. The main role in inducing protolerogenic liver environment is played by KCs, LSECs and pDCs (Figure 6). KC constitutively express TGF-b and PD-1, possess high levels of negative regulators downstream the TLR pathway and secrete IL-10 upon LPS stimulation (Kinoshita et al., 2010; Knolle et al., 1995; Liu et al., 2008; Movita et al., 2012; Roth et al., 1998). TRegs, cytokines: IL-10 and TGF β are the main effectors in inducing tolerance. Kupffer cells and LSECs in response to HBV proteins release IL-10 (Tang et al., 2003), while TGFβ is released by LSECs. IL-10 and TGFβ directly induce TRegs formation. Besides, it is also reported to have direct relation with the pDC and serum DNA levels in CHB (Manigold and Racanelli, 2007) . TRegs also augment IL-10 and TGF β secretion and inhibit CD4 and CD8 function. Moreover, TRegs also suppress the proliferation of autologous PBMCs and their IFN-γ production (Xu et al., 2006). Besides IL-10 also inhibits NK cell effector function and MHC-II expression by macrophages, further reducing HBV specific adaptive cell activation (Dunn et al., 2009; Gordon, 2003). Intrahepatic CTL function is also modulated by co-inhibitory molecules such as PD-1 ligands expression on infected hepatocytes (Fisicaro et al., 2010; Maier et al., 2007; Muhlbauer et al., 2006), which are expressed in response to IFN-α or IFN-γ production (Fisicaro et al., 2010). Intrahepatic CTL function might also be modulated by change in microenvironment nutrient composition; such as the deprivation of T cells of arginine which is observed due to increases in arginase activity accompanying a surge in macrophage population. Arginine is essential for CTLs pro-inflammatory phenotype (Das et al., 2008). Thus there is enough evidence to regard HBV as capable of immunemodulating innate immune response. The outcome of HBV infection is determined by the kinetics of viral spread against the kinetics of innate immune response to limiting infection and timely transition to adptive immune activation.

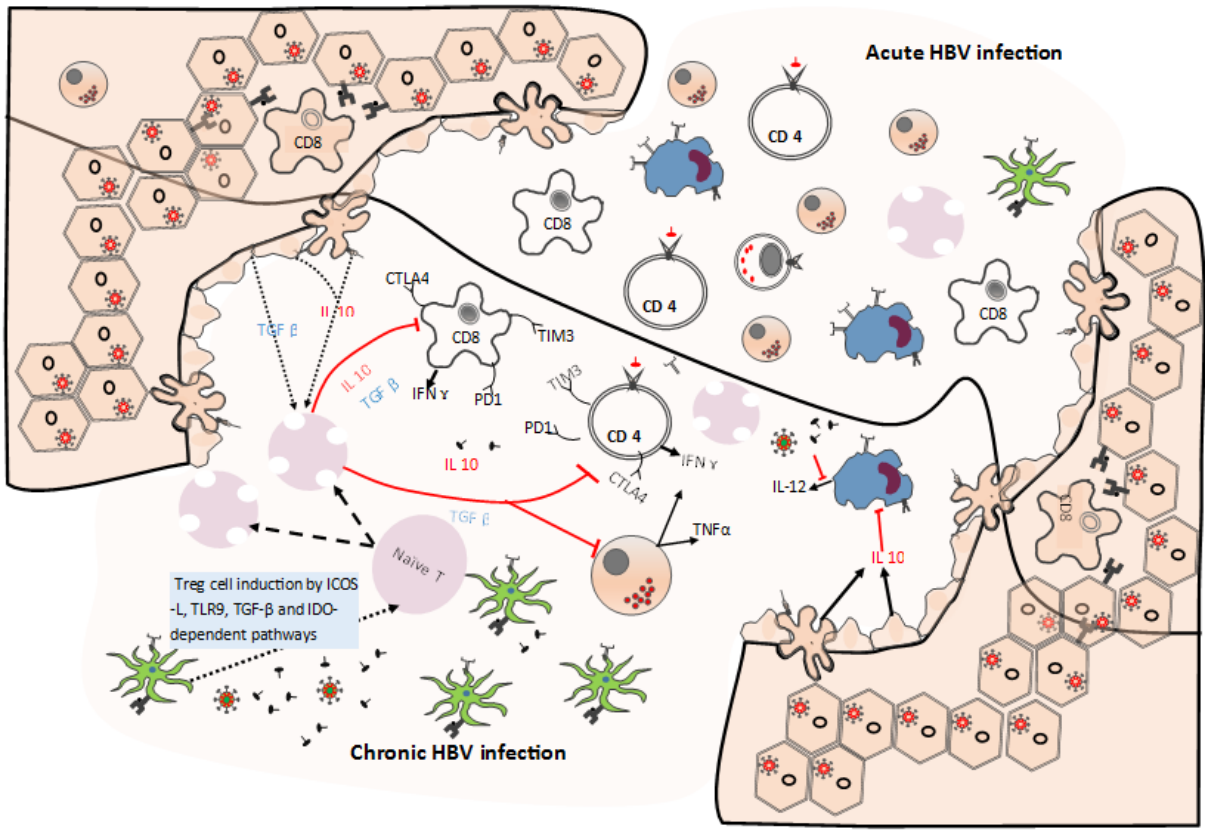
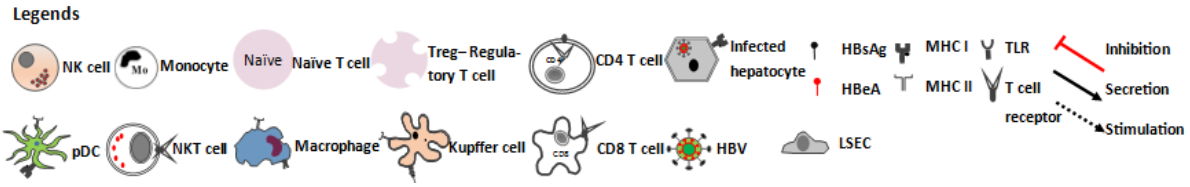


Fig. 6 Immune suppressive niche created by HBV in CHB. Number of TReg cells increase with the increase in pDC. MHC class II molecules are down regulated on macrophages in CHB as compared to during acute HBV infections. CD4 and CD8 cell number and function is also reduced in CHB. **Note :** for microenvironment of liver in acute HBV infection refer **Fig. 2**



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