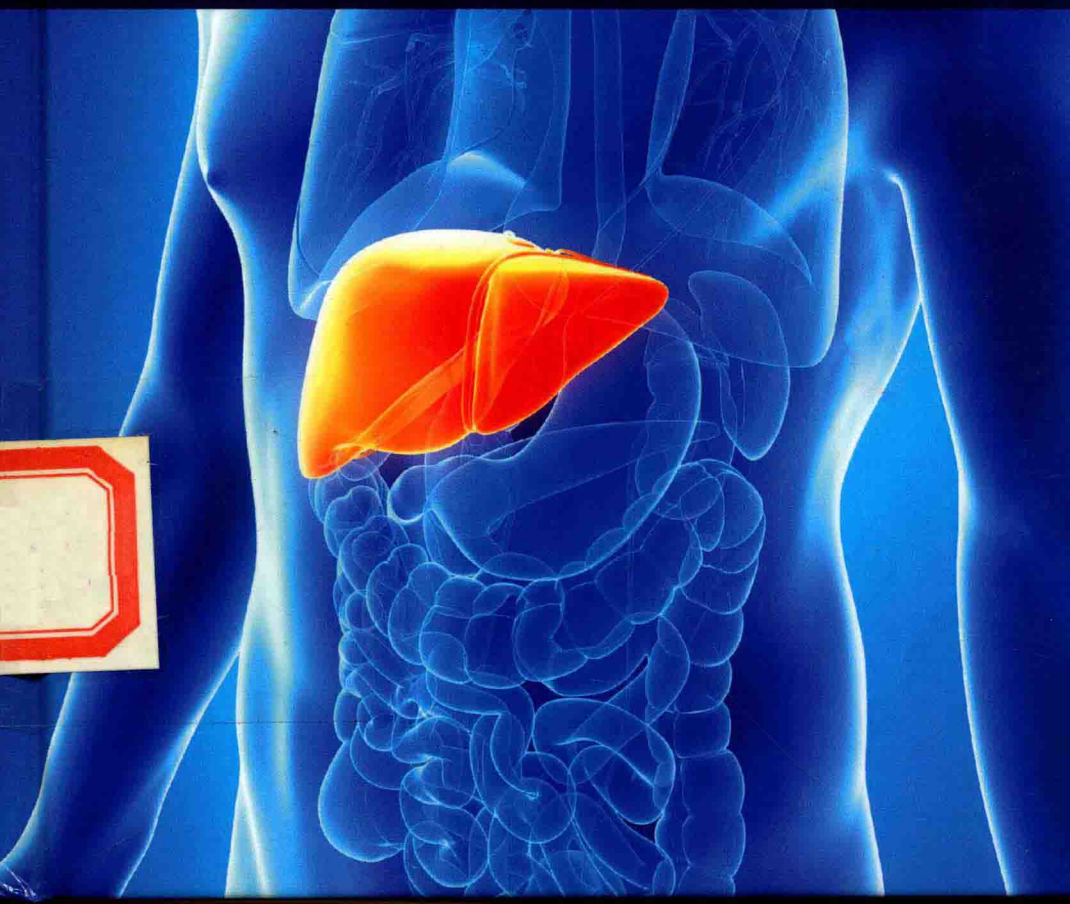
Viral Hepatitis

Mechanism and Diagnosis

Amelia Foster



Viral Hepatitis: Mechanism and Diagnosis

Edited by **Amelia Foster**

New Jersey

Published by Foster Academics,
61 Van Reypen Street,
Jersey City, NJ 07306, USA
www.fosteracademics.com

Viral Hepatitis: Mechanism and Diagnosis
Edited by Amelia Foster

International Standard Book Number: 978-1-63242-423-5 (Hardback)

Printed in China.

Preface

The main aim of this book is to educate learners and enhance their research focus by presenting diverse topics covering this vast field. This is an advanced book which compiles significant studies by distinguished experts in the area of analysis. This book addresses successive solutions to the challenges arising in the area of application, along with it; the book provides scope for future developments.

This book primarily focuses on the elucidation of the mechanism as well as diagnosis of viral hepatitis. There is a wide range of issues related to viral hepatitis studies which need to be dealt with. These include treatment, molecular biology of viruses, epidemiology, laboratory diagnostics, etc. Consequently, a diverse range of special textbooks and monographs have been published on this subject. Considering this fact and the rapid growth in our cognizance of the problem, this book emphasizes on some of the most crucial aspects related to the problem of immune pathogenesis of parenterally transmitted viral hepatitis and some aspects of hepatitis diagnostics. Several groups of researchers have shared vital information and results of studies through this book for the reference of specialists working in the field and readers keen on learning about viral hepatitis. Bruce A. Beutler and Jules A. Hoffmann were awarded by The Nobel Prize Committee (in the field of physiology and medicine, 2011) for their discoveries regarding the activation of innate immunity while Ralph M. Steinman was awarded the same for his discovery of the dendritic cell and its role in adaptive immunity. This book is updated with these discoveries and elucidates the challenges posed by inborn and adaptive immune response in case of viral hepatitis.

It was a great honour to edit this book, though there were challenges, as it involved a lot of communication and networking between me and the editorial team. However, the end result was this all-inclusive book covering diverse themes in the field.

Finally, it is important to acknowledge the efforts of the contributors for their excellent chapters, through which a wide variety of issues have been addressed. I would also like to thank my colleagues for their valuable feedback during the making of this book.

Editor

Contents

1

HBV & HCV Immunopathogenesis

Megha U. Lokhande, Joaquín Miquel, Selma Benito and Juan-R Larrubia
Translational Hepatology Unit, Guadalajara University Hospital, University of Alcalá
Spain

1. Introduction

Hepatitis B and C (HBV&HCV) viruses are two hepatotropic non-cytopathic viruses able to evade immune system efficiently as mechanism to persist in infected hosts. To fight against a viral infection the host displays two kinds of immune responses: the innate and adptive responses. The innate response is the first immunological barrier and it is essential in cytopathic viruses. This response limits viral spreading but also acts as adaptive response activator through antigen presentation to viral specific cells. Adaptive response is the second line in the immunological defense. It plays a major role in non-cytopathic viral infections because this type of viruses behaves as an intracellular parasite and they remain occult to the innate system.

1.1 General features of Innate Immune response

The liver is a unique anatomical and immunological site in which antigens-rich blood from the gastrointestinal tract is passed through a network of sinusoids and scanned by antigen-presenting cells and lymphocytes. It is selectively enriched in macrophages (Kupffer cells), natural killer cells (NK) and natural killer T cells (NKT) which are key components of the innate immune system (Racanelli & Rehermann, 2006).

Innate immunity generally plays a role immediately after infection to limit the spread of the pathogen and to activate the adaptive immune response (Guidotti & Chisari, 2006). Complex interplay between innate and adaptive immunity is the key for the resolution of acute infections. Innate response is induced after host recognition of common molecular patterns expressed by viruses, immediately after primoinfection, and providing a mandatory environment for triggering efficient adaptive immune responses. During hide and seek game of virus and host, one or more viral products get exposed and recognized by early immune response. This starts anti-viral control through direct cytopathic mechanisms (Koyama *et al.*, 1998), antiviral effect by producing IFN type I (IFN-alpha/beta) by infected cells (Samuel, 2001), and activation of the cellular component of the innate immune system as natural killer (NK) cells and natural killer T (NKT) cells (Biron *et al.*, 1999).

Production of type I IFNs can be triggered directly by virus replication through cellular mechanisms that detect the presence of viral RNA or DNA (Alexopoulou *et al.*, 2001), while NK cells are activated by the recognition of stress-induced molecules and/or the modulation of the quantity of major histocompatibility complex (MHC) class I molecules on the surface of infected cells (Moretta *et al.*, 2005).

NK and NKT cells can be rapidly recruited to the site of virus infection and have the potential to recognize infected cells before MHC class I expression is significantly induced on the cell surface. Activated NK and NKT cells may participate in disease pathogenesis directly, by killing infected cells, and indirectly, by producing soluble factors that have antiviral activity, recruiting inflammatory cells into the infected tissue and shaping the adaptive immune response (Biron *et al.*, 1999).

1.2 General features of adaptive immune response

Non-cytopathic viruses behave as intracellular parasites which are hidden to the immune system. They are not usually highly infectious but produce long-lasting diseases that allow them to spread the infection along the time. The host-virus relationship is a dynamic process in which the virus tries to decrease its visibility, whereas the host attempts to prevent and eradicate infection with minimal collateral damage to itself (Nowak & Bangham, 1996).

To control non-cytopathic viral infections, it is necessary the activation of the adaptive immune system, and especially the cellular immune response. Naïve specific CD4+ and CD8+ T cells are primed by dendritic cells in the lymph nodes. Once these cells become activated, they change the phenotype into effector cells and migrate to the infected tissue, attracted by the chemokines produced by the parenchymal cells. Primed specific CD4+ cells are essential to allow the adequate activation of specific cytotoxic T cells by secretion of Th1 cytokines (Larrubia *et al.*, 2009a). This is very important because specific cytotoxic T lymphocytes play a major role in spontaneous infection resolution. These cells are able to recognize the infected cells and to destroy them by cytolytic mechanisms, but they also produce type-1 cytokines that eliminate the virus without producing tissue damage (Fig.-1).

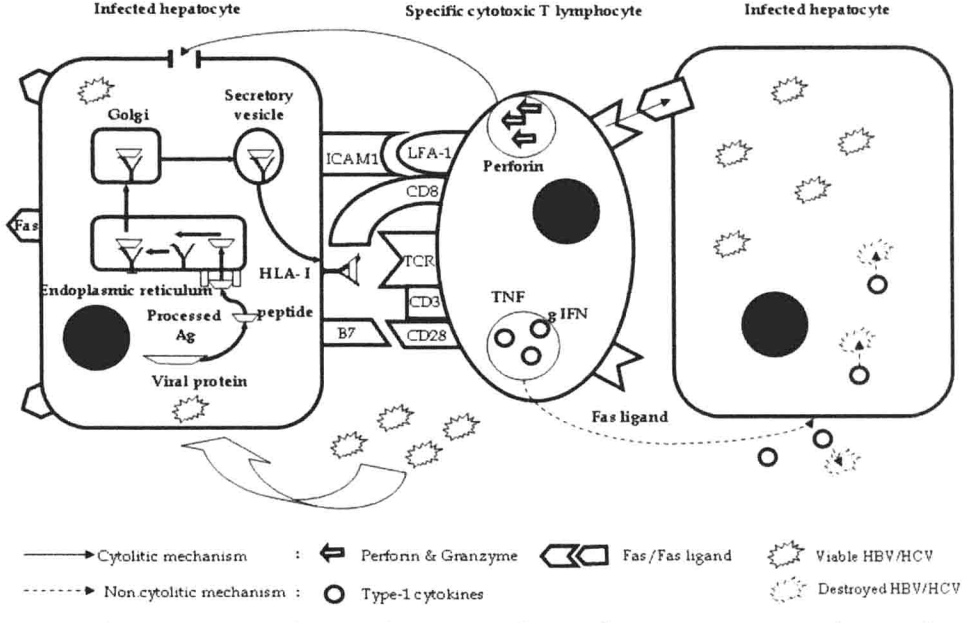


Fig. 1. Cytolitic and non-cytolitic mechanisms to destroy hepatotropic viruses by specific cytotoxic T cells

Both CD4+ and CD8+ cell activation depends on the engagement between T cell receptor and the MHC molecule/epitope complex plus the interaction between co-stimulatory molecules and their ligands (Choudhuri *et al.*, 2005). When these cells have finished their effector task, they express negative co-stimulatory molecules and pro-apoptotic factors to switch-off their activity, and a subsequent constriction in the specific T cell population is produced. After this event, a memory T cell population is maintained for decades to respond faster to a new infection, and in certain cases to keep under control viral occult infection (Appay *et al.*, 2008).
In this chapter the specific features of the immune response against two hepatotropic non-cytopathic viruses (HBV&HCV) able to induce a persistent infection in human are reviewed.

2. HBV immunopathogenesis

HBV is an enveloped incomplete circular double strand DNA virus. This virus is spread around the world and more than 2 billion people have markers of current or past HBV infection, developing chronic infection in approximately 350 million people. Approximately a quarter of persistent infection patients will develop terminal liver disease. The infection is acquired by parenteral, vertical and sexual transmission, and although there is an efficient vaccine, this infection is still an overwhelming health problem, especially in developing countries. Natural HBV control is based on a competent immune response but this is not obtained in 5-10% of infected adults and up to 95% of newborns from HBeAg-positive mothers (Liaw *et al*, 2010). Currently, there are different effective treatments able to control HBV replication but they are not very efficient in inducing either HBeAg or HB surface (HBsAg) Ag seroconversion (Perrillo *et al*, 2010). For this reason, it is interesting to understand the HBV immunopathogenesis to develop immunomodulatory strategies to restore an efficient anti-HBV immunoresponse.

2.1 Life cycle of HBV

Hepatitis B virus (HBV) is not directly cytopathic for the hepatocyte. During the early phase of HBV (before virus-specific T cells enter into the liver), there is no histological or biochemical evidence of hepatocyte damage (Guidotti *et al.*, 1999). Moreover, when cellular immune responses are deficient or pharmacologically suppressed, HBV can replicate at high levels in the liver in the absence of detectable pathological consequences (Ferrari *et al.*, 2003; Wieland *et al.*, 2000). These results suggest that hepatocyte damage during HBV infection is an immune-mediated event. Therefore, this virus is capable to enter, replicate and spread in human hepatocytes without causing any direct damage.
HBV is able to attach to the hepatocyte in a non-cell-type specific manner through cell-associated heparan sulphate proteoglycans. Later, the virus binds irreversibly to an unknown hepatocyte-specific preS1 receptor. After that, two different entry pathways have been proposed: endocytosis and fusion. Finally, the cytoplasmic release of the viral nucleocapsid, containing the relaxed circular partially double stranded DNA (rcDNA), is performed. Then, the nucleocapsid with the rcDNA is transported to the host cell nucleus (Kann *et al.*, 2007). Once rcDNA enters into the nucleus is repaired to complete the double strand DNA to produce the covalently closed circular DNA (cccDNA). The cccDNA stays stable in the hepatocyte nucleus for decades, and it is organized as chromatin like structure (minichromosome) (Levrero *et al.*, 2009). The cccDNA utilizes the cellular transcriptional machinery to produce all viral RNAs necessary for protein synthesis and viral replication.

From an immunological point of view, the cccDNA is extremely important since it will persist in most of the hepatocytes and it is not possible for the immune system to destroy it. For this reason, even if the immune response is able to control HBV infection, it does not mean HBV eradication because cccDNA persists as occult HBV infection in the hepatocytes (Larrubia, 2011; Rehermann *et al.*, 1996). From the pregenomic HBV RNA reverse transcription is performed by HBV DNA polymerase. This new HBV DNA can be either re-imported into the nucleus to form additional cccDNA molecules or can be enveloped with HBV translated proteins for secretion (Urban *et al.*, 2010).

2.2 HBV acute infection

2.2.1 Innate immune response during acute HBV infection

During HBV primo-infection, replication can be efficiently limited by type I IFNs (Wieland *et al.*, 2000; McClary *et al.*, 2000). Nevertheless, data on acutely infected chimpanzees have shown a lack of detection of genes associated to innate response in the liver during the entry and expansion phase of HBV (Wieland *et al.*, 2004). During this phase, HBV can replicate unchecked to extremely high levels. It has been proposed that, because HBV replicates within nucleocapsid particles, viral replicative intermediates of single-stranded RNA or viral DNA, which are strong activators of type I IFN genes, are protected from cellular recognition (Wieland & Chisari, 2005).

Such early events are difficult to analyze during natural infection in humans, because HBV-infected patients are mainly detected after clinical hepatitis, which occur 10-12 weeks after infection. Nevertheless, it is interesting to note that the lack of early symptoms (such as fever and malaise) in HBV-infected patients, typical of other human viral infections, constitutes an indirect evidence of the defective type-I IFN production during the early phases of HBV infection.

In a cohort of patients, sampled in the pre-clinical phase and followed up to infection resolution, serum concentrations of IFN-alpha remained barely detectable during the early incubation phase and throughout the peak of viral replication and subsequent viral load reduction. Circulating IFN-alpha levels in patients with acute HBV infection at the time of peak of viremia were no significantly greater than at the time of infection resolution. Similarly, IFN-kappa and IL-15, which are important for induction of NK effector function, were not induced during the peak of viremia (Dunn *et al.*, 2009).

Consequently, HBV can be considered as a "stealth virus", capable of sneaking through the front line of host defenses. It is possible that this situation of immune suppression might be activated by HBV replication. IL-10 is a potent immunosuppressive cytokine that can inhibit both innate and adaptive immunity. In fact, a close correlation between circulating IL-10 and HBV-DNA levels have been observed. IL-10 increased early in the course of infection, in parallel with the rapid increase in HBV viral load and antigenaemia and before the onset of inflammation. Moreover, the reduction of IL-10 coincided with either the termination of viremia or with HBeAg seroconversion. Consequently, there may be an active suppression of NK responses mediated for IL-10. In further support of this, addition of exogenous IL-10 during in-vitro experiments was able to suppress NK cell IFN-gamma production which was recovered upon blocking IL-10 and its receptor (Dunn *et al.*, 2009).

Although no induction of type-I interferon is observed, within hours after HBV infection, there is a transient release of IL-6 and other proinflammatory cytokines (IL-8, tumour necrosis factor (TNF) alfa, IL-1B). The IL-6 released was shown to control HBV gene

transcription and replication in hepatocytes shortly after infection, ensuring an early control of virus replication, thereby limiting the activation of the adaptive immune response and preventing death of the HBV-infected hepatocytes in the early phases of infection (Hosel *et al.*, 2009). The production of IL-6 and other cytokines seems transient after HBV infection. Interestingly, HBV replication tends to increase 3-4 days after infection, when IL-6 level has returned to baseline. This may suggest that the virus actively counteracts the action of IL-6, like occurs during the human cytomegalovirus infection (Gealy *et al.*, 2005).

However, a role for the innate immune response in the control of early HBV replication should not be dismissed. A study performed in woodchucks (Guy *et al.*, 2008) observed a NK and NKT cell response within hours after inoculation with a liver-pathogenic dose of woodchuck hepatitis virus. These immune responses were at least partially capable of limiting viral propagation but were not followed by a prompt adaptive T cell response, which was delayed for 4-5weeks. Chimpanzees able to control the virus show a typical acute phase of disease with a robust activation of IFN-gamma, and TNF-alpha (Guidotti *et al.*, 1999). It is possible that this initial host response to HBV is primarily sustained by NK and NKT cells, that are capable to inhibit HBV replication in-vivo (Kakimi *et al.*, 2000), as shown by the early development of NK and NKT responses in healthy blood donors who became hepatitis B surface antigen and HBV DNA positive (Fisicaro *et al.*, 2009). Also, an early activation of NK and NKT cells in a woodchuck model of acute hepatitis B infection has been shown. In this model NK and NKT cells induced a transient, but significant reduction of virus replication (Guy *et al.*, 2008).

In human, a study performed in two seronegative blood donors who became positive for HBsAg and HBV DNA, who were monitored throughout very early stages of infection, demonstrated that the human innate immune system is indeed capable of sensing HBV early after infection and of triggering a NK/NKT cell response to contain HBV infection and to allow a timely induction of adaptive response (Fisicaro *et al.*, 2009).

Therefore, rather than being silent, hepadnaviruses may be efficient at counteracting the actions of the innate immune system early after infection. There is a growing body of evidence suggesting that HBV could inhibit innate responses by regulating the expression of Toll-like receptors (TLRs), which are major sensors of viral infection in immune-specialized and non-specialized cells (Barton, 2007). HBV is able to suppress toll-like receptor-mediated innate immune response in murine parenchymal and non-parenchymal liver cells (Wu *et al.*, 2009). Indeed, the expression of TLR1, TLR2, TLR4 and TLR6 is significantly lower in peripheral blood mononuclear cells (PBMC) and hepatocytes from chronic hepatitis B (CHB) patients (Chen *et al.*, 2008). Furthermore, flow cytometric analysis has shown that the expression of TLR2 in PBMC, from CHB patients is significantly decreased. TLR2 expression on PBMC has been correlated with the HBsAg plasma levels (Riordan *et al.*, 2006) and HBeAg protein (Visvanathan *et al.*, 2007). Recently, an immunomodulatory role of HBeAg on innate immune signal transduction pathways, via interaction and targeting of TLR-mediated signalling pathways, has also been shown (Lang *et al,.*2011).

Moreover, dendritic cells (DC) exhibit functional impairment in hepatitis B virus carriers. Plasmocytoid (p)-DC are the major type-I interferon producing cells and sensors of viral infections because they express both TLR7 and TLR9 that respectively recognize, even in absence of viral replication, single-stranded RNA and unmethylated cytosine-guanosine dinucleotide motifs (Fitzgerald-Bocarsly *et al.*, 2008). A recent study reported that, in CHB patients, there was a reduction of TLR-9 expressions in pCDs, which correlates with an impaired IFN-alpha production by these cells (Xie *et al.*, 2009).

Altogether, these data suggest that HBV infection can alter innate immune responses, triggered by both specialized cells and hepatocytes, through down-regulating functional expression of TLR. Currently, whether HBV is a stealth virus for the innate immune response or is able to block it efficiently is a matter of debate.

2.2.2 Adaptive response during acute HBV infection

Despite of the lack of proper innate response activation, this does not affect to adaptive response during HBV primo-infection. HBV-specific T cell response appears soon after the exponential HBV replication phase (Webster *et al.*, 2000). Both, CD4+ and CD8+ specific responses are present and they are polyclonal, vigorous and multi-specific, when the viral control is obtained, while these responses are impaired when the infection progresses over chronicity (Maini *et al.*, 1999). HBV control is achieved through the labor of HBV-specific CD8+ T cells. These cells are able to recognize infected hepatocytes and to destroy them by apoptosis, but also they produce type-I cytokines, such as gamma-interferon and TNF-alpha, which are capable of non-cytopathic HBV clearing (Ferrari *et al.*, 2003; Guidotti & Chisari, 2001). This response to become fully activated needs the adequate stimulation by professional antigen presenting cells and the correct regulation by specific CD4+ cells. HBV-specific CD8+ T cells are responsible of HBV control, but they also initiate a minor liver damage. In fact, most HBV DNA is eliminated by non-cytolitic pathways before amino-transferases elevation is detected. Nevertheless, the secreted IFN-gamma by these cells, in addition to the chemokines produced by infected hepatocytes, attracts non-specific mononuclear cells and polymorphonuclear cells, which are responsible of liver damage amplification (Guidotti & Chisari, 2006). This phenomenon is also acting in the pathogenesis of chronic disease. Specifically, during persistent infection, the HBV specific response is impaired and unable to control the infection, but the hepatocytes continue secreting chemokines to attract effector T cells. However, non-specific inflammatory cells are also attracted and they are the cause of the low grade of persistent liver damage (Bertoletti & Maini, 2000).

During the acute phase of infection, antibodies (Ab) against HBsAg, HBeAg and core (HBc) Ag are produced by activated B cells. HBsAb and HBeAb production is T helper dependent, while HBcAb secretion is dependent and non-dependent from T helper action (Milich & Chisari, 1982). HBs antibodies are produced very early after infection, but they are not detected because they generate complexes with circulating antigens, and therefore they are not detected until the virus is controlled. HBs antibodies prevent viral spreading from one to another hepatocyte and also block circulating HBV. The detection of these antibodies means HBV control and confers natural immunity against re-infection. Observation of HBsAb occurs when HBV is controlled by immune system, and these are neutralizing Abs that will avoid HBV re-infection in case of a new encounter with the virus. HBc Abs are not neutralizing and they indicate HBV contact. When HBc IgM subtype is positive it means acute infection or HBV flare-up during chronic infection. HBe Abs appear before HBs Abs during acute HBV recovery and also when chronic patients shift from a replicative to a non-replicative phase. Moreover, HBe Abs are also present during the HBV chronic replicative phase, when the infecting virus displays a pre-core mutation that avoids HBe Ag production (Maruyama *et al.*, 1994; Milich & Liang, 2003).

During adulthood, most of acute HBV infected cases recover and develop natural immunity due to the combination of a polyclonal, vigorous and multispecific cytotoxic and helper

response (Guidotti & Chisari, 2006). After a self-limited infection, a T cell response constriction is observed and a central memory T cell population is generated. In these cases, a long-lasting protective T cell response is developed. These cells keep under control the intrahepatic HBV traces for decades. In fact, in HBV recovered patients it is possible to demonstrate a T1 orientated multispecific cytotoxic and helper response, decades after primo-infection, and those responses are associated with the observation of HBV DNA in sera or PBMC using ultra-sensitive PCR techniques. These data show that HBV recovery does not mean HBV eradication, since despite of clinical recovery it is possible to demonstrate HBV viral traces that are maintained under control due to the adaptive memory immune response (Larrubia, 2011; Penna *et al.*, 1996; Rehermann *et al.*, 1996).

2.3 HBV chronic infection

Around 5-10% of HBV primoinfection progresses to chronicity in adult infection, while it reaches 95% of newborns from HBeAg-positive mothers and approximately 50% during childhood infection (Liaw *et al.*, 2010). The development of a persistent HBV infection is based on a failure of HBV-specific response due to the induction of an anergic and pro-apoptotic status on this response because of the high viral pressure (Maini *et al.*, 2000a; Webster *et al.*, 2004). Several mechanisms have been involved in the impairment of specific T cell response. Specific T cells behave as anergic cells with progressive impairment of type-1 cytokine production, such as IL-2, IFN-gamma and TNF-alpha. The cytotoxic T cells are neither able to proliferate nor to kill infected hepatocytes after antigen encounter. Nevertheless, cytokines and chemokines produced in the infected liver are able to attract a non-specific inflammatory population causing the persistent liver damage. Several mechanisms are used by HBV to induce this anergic status, which will end-up in a pro-apoptotic situation that could cause specific T cell deletion. Persistent high HBs antigenemia, massive production of defective viral particles and the toleraising liver environment induces an anergic condition on T cells. In fact, HBV infected liver is depleted in tryptophan and there is an accumulation in its toxic metabolite (IDO) which is able to induce immunotolerance (Larrea *et al.*, 2007). Also, arginase I activity is increased during HBV infection provoking an arginine depletion on T cells which causes a CD3ζ down-regulation. The effect of CD3ζ lower expression translates into IL-2 production impairment by HBV-specific CD8+ cells (Das *et al.*, 2008). Interestingly, in the HBV infected liver is increased the secretion of immunosuppressive cytokines. IL-10 is produced by dendritic cells and Kupffer cells while transforming growth factor-beta (TGF-β) is secreted by stellate cells. The level of these cytokines correlates with HBV disease activity during chronic and acute infection (Dunn *et al.*, 2009). Other escape mechanisms involve TRAIL-mediated deletion of HBV-specific CD8+ cells by NK cells (Dunn *et al.*, 2007). Moreover, regulatory T cells can cause HBV-specific T cell activity suppression (Furuichi *et al.*, 2005). On the other hand, persistent HBV infection favors the up-regulation of pro-apoptotic molecule Bim. This molecule mediates premature HBV specific cytotoxic T cell death following intrahepatic antigen presentation (Lopes *et al.*, 2008). Another common mechanism, induced by HBV to evade immune system, is the induction of negative co-stimulatory molecules such as CTLA-4, PD-1, Tim3 and Lag3. Excessive co-inhibitory signals drive T cell exhaustion during chronic HBV-infection (Maini & Schurich, 2010). Finally, HBV is also able to evade specific immune response by developing escape mutation at cytotoxic and helper immunodominant epitopes (Maini *et al.*, 2000b).

2.3.1 Adaptive response during chronic HBV infection

Chronic evolving infection is characterized by several progressive phases with different adaptive response features. The first stage is called immunotolerant phase. This is typical for countries with high rates of mother to child HBV transmission, but it is not seen in Western countries, where this route of transmission is not common. During this phase, HBV viral load is extremely high, but the liver damage and the anti-HBV immune response are absent. Several studies from D. Milich group, in HBe+ transgenic mice, have shown that the lack of HBV-specific immune response is due to some properties of HBeAg. This viral protein is able to cross the placenta to reach the offsprings thymus, where this is considered a self-antigen, eliciting HBe/HBc Ag-specific T helper cell tolerance in uterus (Milich *et al.*, 1990). Moreover, during this phase, high HBV viral load inhibits adaptive immune response. In fact, frequency and function of HBV-specific T cells is inversely correlated with HBV viral load (Boni *et al.*, 2007; Webster *et al.*, 2004) (Fig.-2). In the natural history of chronic HBV infection, this phase is followed by the immuno-clearance stage. This is the common starting point in persistent infection in Western countries. This phase is characterized by viral replication and liver damage fluctuations. Even though the specific immune response is quite inefficient, it is still able to obtain certain HBV control. During this phase, HBeAg seroconversion and HBV pre-core mutant selection is possible. HBe seroconversion allows the change to another HBV infection phase with a higher viral control and lower liver damage. HBe seroconversion is faster in individuals with certain polymorphisms at IL-10 and IL-12 genes. In these cases, high levels of IL-10 and IL-12 are observed and they are a predictor of spontaneous HBe seroconversion (Wu *et al.*, 2010). Another typical feature of the immuno-clearance phase is the presence of HBV exacerbations, characterized by HBeAg level increase followed by transaminase level raise. The HBeAg level increase induces an activation of HBc/HBe specific response activation, after this a decrease in HBeAg and transminase level is observed, followed by a specific T cell response constriction. This data show that HBV-specific T cell activation due to HBeAg level is causing acute exacerbations in HBeAg+ chronic patients (Frelin *et al.*, 2009). This phenomenon can lead to liver damage generation, HBe seroconversion and pre-core mutant selection. During these HBV acute exacerbations, HBV-specific cytotoxic T cells destroy wild-type HBV infected hepatocyte producing liver damage. Moreover, if along this stage HBV pre-core mutants emerge, these cytotoxic T cells can select them, since the infected hepatocytes with these variants are not recognized properly by cytotoxic T cells. In fact, liver infected cells by the wild type virus are eliminated more efficiently by specific cytotoxic T cells than cells infected by the pre-core mutant. This is because wild-type infected cells presenting HBc and HBe epitopes are better targets for cytotoxic T cells than cells infected by HBV pre-core mutant expressing only core epitopes (Frelin *et al.*, 2009). This situation leads to HBe antigen negative form of chronic hepatitis B with persistent liver damage, which is different to the wild-type HBe seroconversion where the infection can be consider inactive. This last one is the third phase of the chronic HBV natural history which is called low or non replicative phase, and corresponds to the clinical inactive carrier state. In this stage viral load and liver damage is very low. During this phase HBV-specific T cell responses are present and are quite efficient despite lack of liver damage. These cells are very competent in controlling infected hepatocytes, preventing HBV spreading and the development of liver infiltration by non-specific inflammatory cells, which are the cause of persistent liver

damage during chronic active hepatitis B. Therefore, it is considered that during the low/non-replicative phase HBV is under a partial control by HBV-specific response (Maini *et al.*, 2000a). At this stage, it is possible to observe HBV reactivation associated with hepatitis flares, mainly in the case of infection by HBV pre-core mutants. This last phase of HBV natural history is called reactivation phase. The immunological causes of these reactivations are not very well known yet. During these hepatitis flares is not possible to demonstrate the presence of HBV-specific T cell reactivity, but it is observed NK cell activation which correlates with the degree of liver damage (Dunn *et al.*, 2007). Therefore, in this last step of chronic HBV natural history, the innate response could be involved.

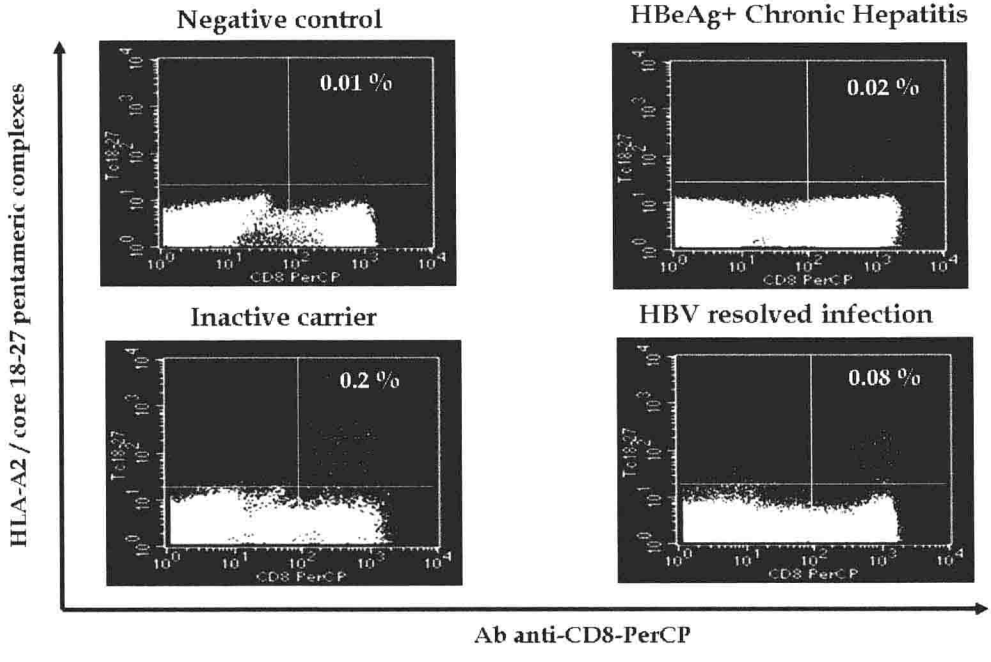


Fig. 2. FACS® dot-plots from peripheral blood mononuclear cells of HBV infected patients with different HBV control stained directly ex-vivo with Ab against CD8 plus multimeric HLA-A2/core 18-27 complexes. A negative correlation between viral control and frequency of HBV-sepcific CD8+ cells is observed. Figures in the upper right quadrant show the frequency of HBV-specific CD8+ cells out of total CD8 population.

In summary, HBV is not ever completely eliminated from the infected host, but there is a gradient of control according to the functional efficiency of HBV-specific response. In patients with HBV natural immunity, they present a HBV occult infection with a very efficient control by CD4+ and CD8+ specific HBV T cells. This immune control is partial in patients in the inactive carrier state and completely inefficient in cases with chronic active hepatitis (Boni *et al.*, 2007; Maini *et al.*, 2000a; Zerbini *et al.*, 2008). Strategies directed to restore anti-HBV adaptive response could help in the permanent infection control.

3. HCV immunopathogenesis

The hepatitis C virus (HCV) is an enveloped; positive stranded RNA virus and represents the Hepacivirus genus in the Flaviviridae family. It has been estimated that more than 170 million people are infected with HCV, since clinical identification and molecular cloning of HCV in late 1980s. This virus is spread by contact with infected blood and body fluids. Approximately 80% of infections succeed in establishing a chronic infection with the potential for developing severe liver diseases such as cirrhosis and hepatocellular carcinoma (HCC) (Lavanchy, 2009; Tsukuma *et al.*, 1993).

No effective vaccine against HCV is available till date. Current standard-of-care therapy for HCV infection as peg-interferon-alpha and ribavirin (Pawlotsky, 2004), has limited efficacy, in particular against the genotype 1 virus (Fried *et al.*, 2002; Manns *et al.*, 2001). An extended search for new therapy is progressing, already passed for marketing authorization of the protease-inhibitors (Poordad *et al*, 2011). A major concern with new therapy is rapid development of drug-resistant viral mutants. Due to the failure or side effect of the treatment, stepping forward for understanding the immunopathogenesis of HCV infection is essential in the development of a therapeutic vaccine and immunomodulatory treatments for chronic infections.

Due to the lack of adequate cell culture systems, HCV studies have been slowed down for a long time, but continuous progress in the last few years it has overcome this obstacle. In-vivo model to study the biology of HCV have been significantly restricted due to the limited experimental availability of chimapanzees, the primary model for HCV (Alter *et al.*, 1978; Bukh, 2004), and difficulties encountered in reproducing true infection in small animals. Two breakthroughs has been an important contribution to the field: firstly, subgenomic replicons (i.e. without structural genes) (Blight *et al.*, 2000; Blight *et al.*, 2003; Lohmann *et al.*, 1999), which are highly permissive for HCV replication (Blight *et al.*, 2002) and secondly, HCV complete replication in cell culture (Lindenbach *et al.*, 2005; Wakita *et al.*, 2005; Zhong *et al.*, 2005). However, it has long been recognized that these models are complicated by the particularly high error rate of the HCV RNA replicase (Rong *et al.*, 2010).

It is widely accepted that immune-mediated host-virus interactions are responsible for the outcome of HCV and pathogenesis of further severe diseases. In this chapter, it is covered how virus evades primary defense mechanisms. Finally, adaptive immune response escape mechanisms induced by HCV to become persistent are also analyzed. To be familiar with pathogenesis of HCV infection, a brief outline of HCV life cycle is provided below.

3.1 Life cycle of HCV

The development of HCV replicons (Blight *et al.*, 2000; Blight *et al.*, 2003; Ikeda *et al.*, 2002; Lohmann *et al.*, 1999), HCV pseudotyped particles (HCVpp) (Bartosch *et al.*, 2003a) and most recently the infectious HCV cell culture system (Lindenbach *et al.*, 2005; Wakita *et al.*, 2005; Zhong *et al.*, 2005) have advanced our understanding of the viral life cycle. Hepatocytes are the primary site of HCV infections. HCV life cycle begins with binding of the virus to cell surface receptors. The putative receptors, the tetraspanin protein CD81 (Bartosch *et al.*, 2003a; Hsu *et al.*, 2003; Pileri *et al.*, 1998; Wunschmann *et al.*, 2000), the scavenger receptor class B member I (SR-B1) (Bartosch *et al.*, 2003a; Grove *et al.*, 2007; Kapadia *et al.*, 2007; Scarselli *et al.*, 2002) and the tight junction proteins claudin-1 (Evans *et al.*, 2007) and occluding, (Benedicto *et al.*, 2009; Liu *et al.*, 2009; Ploss *et al.*, 2009) have all been shown to enable HCV entry. In addition, the low-density lipoprotein receptor (Agnello *et al.*, 1999;

Molina *et al.*, 2007; Monazahian *et al.*, 1999; Wunschmann *et al.*, 2000), asialoglycoprotein receptor (Saunier *et al.*, 2003), and glycosaminoglycans (heparin sulfate) are also involved, but their exact roles have not been determined. By clathrinmediated endocytosis (Blanchard *et al.*, 2006; Meertens *et al.*, 2006), HCV enters the cell. The virus undergoes an uncoating process by fusion between the viral envelope and endosomal membrane in the acidified endosomal compartment (Bartosch *et al.*, 2003b; Haid *et al.*, 2009; Hsu *et al.*, 2003; Koutsoudakis *et al.*, 2006; Lavillette *et al.*, 2006; Tscherne *et al.*, 2006) via E1/E2-mediated class II fusion (Garry & Dash, 2003; Lavillette *et al.*, 2007), to expose the viral genomic RNA to host-cell machinery. About ~9.6 kb viral RNA genome is released into the host cell cytoplasm, to serve as template for the translation of the viral proteins. IRES-mediated translation of the HCV genome produces a single ~3,000 amino-acid polyprotein (Moradpour *et al.*, 2004), which is processed by cellular and viral proteases into at least 10 different protein products. These products include the structural proteins, which form the viral particle (the virus core and the envelope proteins E1 and E2), and the nonstructural proteins P7, NS3, NS4A, NS4B, NS5A and NS5B (Guidotti & Chisari, 2006). Viral replication is driven by minus strand intermediate. HCV double stranded RNA (dsRNA) is freely exposed in the cytoplasm of infected cell (Moradpour *et al.*, 2004), which is recognizable for host innate immune system. Nucleocapsid is formed by assembling capsid proteins and genomic RNA and bud through intracellular membranes into cytoplasmic vesicles. Finally, by secretory pathway, mature enveloped virions release from the cell.

3.2 Innate immune response during acute HCV infection

The first response to HCV protein is thought to be IFN-β production by infected hepatocytes, which are able to secrete type I IFN. The infected cells are sensed with pathogen associated molecular patterns (PAMP), Toll like receptor (TLR3) (Marie *et al.*, 1998) and retinoic acid-inducible gene I (RIG-I) (Bauer *et al.*, 2001; Sato *et al.*, 2000) by endosomal dsRNA and cytosolic dsRNA respectively, which is an essential intermediate in the HCV replication cycle, and thus, they may be important in the pathogenesis of hepatitis C (Saito *et al.*, 2008). RIG-I recruits IFN-β promoter stimulator protein 1 (IPS-1; also called CARD adaptor inducing IFN-β CARDIF), virus-induced signaling adapter (VISA), and mitochondrial antiviral signaling protein (MAVS) (Hoshino *et al.*, 2006; Meylan *et al.*, 2005; Xu *et al.*, 2005), after ATP-driven activity dependant on recognition of viral protein (Honda *et al.*, 2004). On other hand, TLR3 dimerization, due to leucine-rich repeats (Liu *et al.*, 1998), recruits the adapter protein, Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF). Both processes result in downstream signaling, nuclear translocation of IFN regulatory factor 3 (IRF3) and leads to stimulation of the transcription of a set of genes including IFN-β (Kawai & Akira, 2008). Antiviral state, induced by secreted IFN β, gives an alert to uninfected cells by activation of effector molecules. Binding of IFN α-β to cognate receptor complex lead to the activation of JAK/STAT pathway, which results in the induction of IFN-stimulated genes (ISGs) and lead to enhance the IFN response (Rehermann, 2009) (Fig.- 3).

However, HCV has organized a number of countermeasures not only to inhibit the induction phase, but also interfere with the effector phase of the IFN system (Fig.- 3). It has been confirmed, in in-vitro studies, that HCV serine protease, NS3/4A is enable to cleave MAVS (Li *et al.*, 2005b), TRIF (Li *et al.*, 2005a), IPS-1 (Foy *et al.*, 2003) and oligomerization of MAVS, which is part of signaling process (Kawaguchi *et al.*, 2004; Li *et al.*, 2005a; Li *et al.*, 2005b; Marie *et al.*, 1998; Meylan *et al.*, 2005; Sakamoto *et al.*, 2000). Disruption of IRF-3

activation occurred by NS3 protein action (Liu *et al.*, 1999) and it has been shown with different cell lines in-vitro studies (Kawaguchi *et al.*, 2004; Marie *et al.*, 1998). Another key player, HCV core, when over expressed in cell culture, disturbs antiviral activity via interfering in JAK/STAT signaling and ISG expression by inhibition of STAT1 activation. Simultaneously it induces its degradation (Gale & Foy, 2005; Lin *et al.*, 2006) by induction of inhibitor of the JAK/STAT pathway SOCS3 (Bode *et al.*, 2003), protein phosphatase 2A (PP2A), which ultimately reduces the transcriptional activity of ISG factor 3 (ISGF3) (Heim *et al.*, 1999); and inhibition of ISGF3 interaction to IFN-stimulated response elements (Rehermann, 2009). HCV NS5A interferes with the function of ISGs by inhibiting 2'-5' oligoadenylate synthetase (2'-5' OAS) and leads to overall ISG expression impairment (Polyak *et al.*, 2001). Protein kinase R (PKR) can negatively regulate HCV replication noncytolytically in cell cultures (Kim *et al.*, 2004; Zhao *et al.*, 2004), which can interacts with HCV NS5A and lost its function. Interestingly, HCV E2 acts as distraction target to PKR (Taylor *et al.*, 1999). To sum up, the main targets of HCV proteins to evade immune response are interference with the induction of IFN synthesis, IFN- induced intracellular signaling and IFN-induced effector mechanisms (Fig.-3).

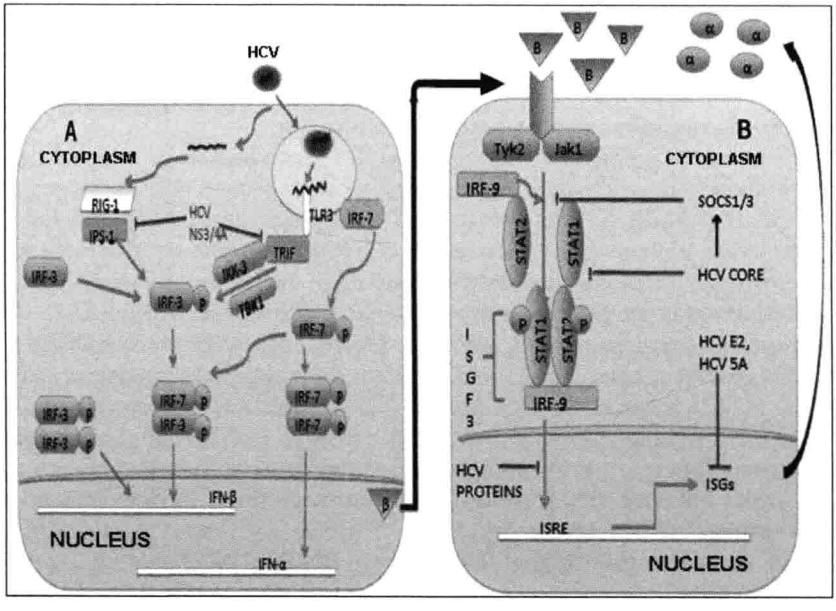


Fig. 3. Evasion of Innate immune response by HCV: (A) Interference in IFN synthesis: Blocking of TLR 3 and RIG-1 signalling respectively, by cleavage of the adaptor molecule TRIF and IPS-1 via HCV NS3/4A; (B) Interference in IFN-induced effector mechanisms: Binding of IFN β and its receptor with TYK2 and JAK1 kinase activation lead to form ISGF3 complex, where this complex interact with IFN stimulated response elements (ISREs) within the promoter and enhancer region of ISGs to induce ISGs (such as 2', 5' OAS, PKR, IRF7) production in nucleus. HCV core induce SOCS1/3, which is the inhibitor of the JAK/STAT pathway and inhibits STAT1 phosphorylation, which inhibits assembly of trimeric ISGFs complex. Function of ISGs is inhibited by HCVE2 and HCV NS3/4A.