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 adaptive 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 4-5 weeks. 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. Plasmacytoid (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 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-1 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 transaminase 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-specific CD8+ cells is observed. Figures in the upper right quadrant show the frequency of HBV-specific CD8+ cells out of total CD8 population.](https://www.intechopen.com)

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;
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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 clathrin-mediated 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.
Dendritic cells (DC) are professional antigen presenting cells with important functions in antiviral immunity through activation of adaptive immune responses. Type-I IFNs are also produced by pDCs, which derive from the lymphoid lineage. Although, production of IFN alpha/beta, in early phase of infection occurs after recognition of ssRNA and dsRNA by TLR7 and TLR9 respectively, the mechanism is still not clear (Albert et al., 2008). The frequency of pDCs in the blood (Nakamoto et al., 2008) and their production of IFN-α in HCV infection is reduced after in vitro stimulation (Bowen et al., 2008). The possible mechanism has been demonstrated in in-vitro studies. First, HCV core and NS3 activate monocytes by TLR2 signaling to produce TNF-α (Izaguirre et al., 2003), which in turn inhibits IFN-α production and induces pDC apoptosis (Bowen et al., 2008). Second, HCV itself inhibits IFN-α production of pDCs (Diepolder et al., 1995). However, other studies revealed regular response to TLR stimulation by circulating pDCs of chronically infected individuals (Decalf et al., 2007; Longman et al., 2005) and they have high levels of endogenous type I IFNs without immuno-dysfuction (Albert et al., 2008). Although this defense mechanism is significant, the host rarely overcomes HCV infection, which suggests several other viral evasion mechanisms that are poorly or not understood yet.

Another group of DCs, myeloid DCs (mDCs) derive from the myeloid lineage (Lanzavecchia & Sallusto, 2001; Steinman et al., 2003). Due to its tolerogenic and stimulatory role (Lanzavecchia & Sallusto, 2001; Steinman et al., 2003), mDCs have been broadly studied in HCV infection. mDCs have not been observed to be decrease in peripheral blood or dysfunctional in HCV chronic infected individuals in in-vitro studies (Kanto et al., 1999; Longman et al., 2004). Nevertheless, HCV proteins can interact with monocytes/macrophages through TLR2, inducing the IL-10 production, which hampers IL-12 production by mDC and IFN-alpha by pDC, or they directly inhibit DC differentiation (Szabo & Dolganiuc, 2005). IL-12 cytokine production by mDC is decreased in HCV patients in response to stimuli like CD40 L or poly (I:C) (Anthony et al., 2004), which can explain clearly the shift from Th1 to Th2 response in HCV patients. In-vitro studies indicates that DC expressing core and E1 proteins have lower stimulatory ability, which is associated to the lack of maturation after stimulation with TNF-alpha or CD40L (Sarobe et al., 2003).

Other cells involved in the innate response are the NK cells. Functions of these cells include generating a cytotoxic response, regulatory cytokines production and control on DC maturation and amplitude of DC response, which may deeply impact on type of downstream adaptive immune responses. Response to HCV infection by NK cell is direct apoptosis induction of infected cells with production of antiviral cytokines (Golden-Mason & Rosen, 2006; Lodoen & Lanier, 2006). Moreover, NK cell depletion or dysfunction favor HCV persistence (Golden-Mason et al., 2008). The role of interactions between HLA class I and killer cell-Ig-like receptors (KIR) during HCV infection has been shown. KIR can regulate NK cell activities. However puzzling contradictions for this topic in different studies have been revealed (Montes-Cano et al., 2005; Paladino et al., 2007; Rauch et al., 2007). The importance of NK cells in the resolution of HCV infection is illustrated by the influence of genetic polymorphisms of KIR and their HLA ligands on the outcome of HCV infection, which was dependent on a homozygous HLA class I ligand background (Khakoo et al., 2004; Knapp et al., 2010; Stegmann et al., 2010). There is need to focus on clear understanding of functional and molecular HLA-KIR interactions to know about the possible way for NK cell-mediated protection in animal models of HCV infection.
However, an increased proportion of NK cells expressing activating receptors, enhanced cytotoxicity and defective cytokine production have revealed in chronic HCV infection (Oliviero et al., 2009). Megan et al revealed that IL28A cytokine could significantly inhibit IFN-γ production lead to NK cell inactivation (Ahlenstiel et al., 2010), which would be important to attenuate chronically activated NK cells. Consequently, the analysis of functional scene between NK cells and type 3 IFN in the immune response to virus will be required to understand the role of the NK in disease progression during HCV infection.

3.3 Adaptive immune response
The second barrier to control HCV infection is the adaptive immunity. This response has two arms to fight against pathogens; humoral and cellular immune response. Humoral immune response, that means neutralizing and non-neutralizing antibodies can endorse antiviral activity and pathogenesis (Guidotti & Chisari, 2006). Cellular immune response shows antiviral immunity by means of virus specific CD8 cytotoxic T lymphocytes (CTLs) and CD4 T helper cells, which play key effector and regulatory roles respectively. These T cells take part in viral pathogenesis of HCV by direct killing of infected cells or producing soluble factors able to clear the virus in a non-cytolytic manner, but also can lead to HCV pathogenic events, favoring direct liver damage and attracting non-specific inflammatory cells to perpetuate the liver inflammation (Guidotti & Chisari, 2006).

3.3.1 Humoral immune response
Neutralizing antibodies (nAbs) generally play a critical role for controlling initial viremia and protecting from re-infection in viral infections. However, the role of the humoral immune response in the clearance of HCV infection has been in the dark for a long time due to difficulties to determine relative role of antibodies to neutralize HCV. It can exclusively be evaluated by relevant model systems. It is thought that HCV clearance could occur in the absence of nAbs. If they are present alone, these Abs are inadequate to eradicate HCV in most of the cases in early studies (Dustin & Rice, 2007; Lechner et al., 2000a; Lechner et al., 2000b; Logvinoff et al., 2004; Thimm et al., 2002).

It has been proved that HCV specific T cells may compensate for lack of neutralizing antibodies to obtain HCV clearance (Semmo et al., 2006). However, due to the development of novel model systems (Bartosch et al., 2003a; Baumert et al., 1998; Lindenbach et al., 2005; Wakita et al., 2005; Zhong et al., 2005), it is possible to focus on HCV entry into host cells and neutralization process which demonstrated that nAbs are induced by patients who subsequently control (Lavillette et al., 2005) or resolve (Pestka et al., 2007) viral infection in the early phase of infection and contrary in chronic infection. This suggests that a strong, early, broad nAbs response may contribute to resolution of HCV in the acute phase of infection while delayed induction of nAbs may contribute to development of chronic HCV infection.

Instead of the rapid, vigorous and multi-specific antiviral host immune responses, chronic patients have been shown to develop a delayed and inefficient neutralizing antibody response (Pestka et al., 2007) due to HCV escape mechanism (Zeisel et al., 2008). Recent studies evident that entry of HCV can be hampered or modulated by nAbs of chronic HCV patients (Gal-Tanamy et al., 2008; Haberstroh et al., 2008; Keck et al., 2009), while it is controversial in cell culture study (Grove et al., 2008). In addition, although nAbs are incapable to clear the virus in chronic infection, due to selection pressure exerting on viral
variants, they contribute to the evolution of the HCV envelope sequences to escape (Farci et al., 2000; von Hahn et al., 2007). It has been proposed that HCV stimulates B cells in a B cell receptor-independent manner in chronic infection (Racanelli et al., 2006) and may favor the development of lymphoproliferative and autoimmune diseases (Guidotti & Chisari, 2006). Although, in vitro studies evidence that the neutralization ability of HCV-specific nAbs enhanced by complement activation against pseudotyped viruses (Racanelli et al., 2006), there is absence of direct experimental evidence about the presence of any of these Ab-mediated functions during natural HCV infection. However, immune complexes are believed to play a pathogenetic role in the development of manifestations such as cryoglobulinemia, glomerulonephritis, porphyria cutanea tarda, and necrotizing cutaneous vasculitis during chronic HCV infection (Agnello & De Rosa, 2004; Amarpurkar & Amarpurkar, 2002; Manns & Rambusch, 1999).

3.3.2 Cellular immune response

Cytotoxic T lymphocyte (CTL) responses are essential to control HCV infection. Efficiency of antiviral CTL responses depends on where these cells are primed. Efficient antiviral CTL response is observed when it is primed in lymphoid organs, whereas within the liver, priming is more tend to induce T cell inactivation, tolerance or apoptosis (Guidotti & Chisari, 2006). A strong, multispecific and long-lasting T-cell immune response emerge to be important for control of viral infection (Dustin & Rice, 2007; Zhang et al., 2009). Persistent HCV unsuccessfully control by T effector cells is due to multiple causes, such as: HCV escape mutant generation, immunosuppressive effects exertion, Tregs induction, or effector T cell exhaustion or apoptosis (Bassett et al., 1999; Thimme et al., 2006; Thimme et al., 2001; Larrubia et al., 2011).

3.3.2.1 Adaptive cellular response during acute HCV infection

Vigorous CD4+ and CD8+ T cell responses targeting multiple HCV regions with intrahepatic production of IFN-γ emerged in acute hepatitis C infection (Bowen & Walker, 2005; Lechner et al., 2000b; Thimme et al., 2001). Decreasing viral titer correlates precisely with the appearance of HCV-specific T cells and IFN-γ expression in the liver (Shin et al., 2006). The appearance of HCV-specific T cells can be detectable in the peripheral blood or in the liver compartment several weeks after infection in humans or experimental chimpanzee models (Dustin & Rice, 2007; Rehermann, 2009), respective with primary peak of transaminases and irrespective of clinical outcome (resolution or chronicity). Delayed emerging of antigen-specific responses are also essential for the HCV control (Rehermann, 2009).

The protective function of CD4+ T cells appear to be due to the production of antiviral cytokines, but also their helping nature to antiviral B cells and in maintenance of CD8+ T cell memory. The HCV clearance has been observed and correlated with vigorous proliferation of specific CD4+ T cells (Diepolder et al., 1995; Missale et al., 1996) with concurrent IL-2 and IFN-γ production (Kaplan et al., 2007; Urbani et al., 2006a). The early sustained development of CD4+ T cell response needs to be successful for viral clearance (Urbani et al., 2006a). Whereas, HCV-specific CD4+ T cell responses are not observed in chronic HCV infection. Moreover, the recurrent viremia has been correlated with loss of previous strong CD4+ T cell responses after several months of viral clearance (Gerlach et al., 1999; Nascimbeni et al., 2003). Studies on the relative importance of CD4 help in spontaneous recovery in acute HCV infection demonstrated that fact (Smyk-Pearson et al., 2008). CTL priming in presence of CD4 help is critical factor in protective function (Urbani et al., 2006a).
On the other hand, the magnitude of CD8+ T cells response in acute HCV infection does not correlate with the clinical or viral outcome (Francavilla et al., 2004; Kaplan et al., 2007; Urbani et al., 2006a). Expression of a dysfunctional phenotype with weak proliferation, low IFN-γ production, impaired cytotoxicity and increased levels of the well known exhausted phenotype programmed death-1 receptor (PD-1) are found in HCV infection, irrespective of infection progression (Bowen et al., 2008; Keir et al., 2007; Nakamoto et al., 2008; Sharpe et al., 2007; Trautmann et al., 2006; Urbani et al., 2006b; Larrubia et al., 2011). Antigen-dependent reactivity of HCV-specific CD8+ T cells has been proved by a rapid decay of CD8+ T cell responses during antiviral therapy (Rahman et al., 2004). However, the appearance of self-sustaining memory T cells (CD127+ memory HCV-specific CD8+ T cells and CD4+ T cells) are necessary to control HCV infection (Lechner et al., 2000b; Thimme et al., 2001; Urbani et al., 2006a). In fact, years after HCV control due to anti-HCV treatment it is possible to find HCV traces in association with HCV-specific T cell reactivity. These data suggest that HCV-specific memory T cells are essential to clear HCV infection completely after the initial acute clearing (Veerapu et al., 2011).

3.3.2.2 Adaptive cellular response during chronic HCV infection

Complete resolved HCV patients exhibit broader CTL responses with higher functional avidity and wider cross-recognition ability than patients with persistent HCV infection (Yerly et al., 2008). There are evidences that demonstrate rapid mutation in HCV genome, T cell exhaustion because of expression of inhibitory molecules (Fig.-4), immune regulatory cytokine induction and immune modulatory T reg cell activation, which are main reasons for HCV persistence in chronically infected patients (Hiroishi et al., 2010; Pavio & Lai, 2003; Seifert et al., 2004; Tester et al., 2005; Larrubia et al., 2011).

![FACS® dot-plots and histograms](www.intechopen.com)
Like Retrovirus, HCV polymerase has high replication rate and lack of proofreading capacity, which permit a rapid virus escape from emerging humoral and cellular immune responses and lead to persistent infection (Chang et al., 1997; Tester et al., 2005). Mutation study in early HCV infection in HLA class I restricted epitopes targeted by CD8+ T cells are associated with persistence (Ray et al., 2005; Timm et al., 2004), which proved indirectly that HLA-restricted CD8+ T cells exert selection pressure. Furthermore, the HLA alleles can influence infection outcome (Neumann-Haefelin et al., 2006).

The secretion of certain immuno-regulatory cytokines is also related with HCV persistence. IL-10 cytokine is found to increase in chronic HCV infection (Piazzolla et al., 2000). In chronic HCV patients, the suppression of IFN-γ production and proliferation of virus-specific CD4+ and CD8+ T cells have been observed in livers with IL-10-producing HCV-specific CD8+ T cells (Accapezzato et al., 2004). IL-10 produced by monocytes or NK cells downregulates effector T cell responses. For instance, monocytes secrete IL-10 in response to HCV core-mediated TLR2 stimulation in vitro (Dolganiuc et al., 2006). IL-10 producing HCV-specific CD8+ T cells inhibits IFN-α production (Duramad et al., 2003), but also promotes apoptosis of pDCs (Dolganiuc et al., 2006), and induces liver infiltration of chronically infected individuals, suggesting that they modulate liver immunopathology to favor HCV persistence (Accapezzato et al., 2004). In addition, intrahepatic HCV-specific IL-10 producing CD8+ T cells prevent liver damage during chronic disease (Abel et al., 2006). Moreover, TGF-b is also involved in antiviral immune suppression and chronic HCV infection evolution (Alatrakchi et al., 2007). To sum up these data, regulatory cytokines such as IL-10 or TGF-beta decrease liver inflammation, after affecting the protective immune response, developing a dual task. First of all, they impair T cell responses to allow viral persistence but also decrease liver damage to extend host survival.

Regulatory T cells (Tregs) are important to control the balance between host damage and viral control produced by specific immune response. In cases of excessive immune response, that could be harmful for the host, these cells can induce immune-tolerance to the viral epitopes. Tregs are derived from natural or induced T cell populations, in which natural CD4+ Tregs are generated during normal T cell development in the thymus, whereas induced Tregs are generated from mature T cells (Bluestone & Abbas, 2003). T cell subset with suppressive function, CD4+ CD25+ FoxP3+ regulatory T (Treg) cells, engages in the control of autoimmunity and immune responses, through various mechanisms including the inhibition of APC maturation and T-cell activation (Shevach, 2009). No difference has been found in the frequency of Treg cells and the extent of suppression irrespective of the outcome of the infection (Manigold et al., 2006). However, higher Tregs frequency has been observed in chronic HCV infected patients than in resolved patients (Boettler et al., 2005; Cabrera et al., 2004; Rushbrook et al., 2005; Sugimoto et al., 2003). Interestingly, depletion of CD25+ cells could enhance responsiveness of the remaining HCV-specific effector cells in vitro (Boettler et al., 2005; Cabrera et al., 2004; Sugimoto et al., 2003), which suggest a fundamental role of Tregs in the establishment of chronic HCV infection. Moreover, Treg cells are induced and proliferate in chronic HCV infection and appeared to alter liver inflammation (Zerbini et al., 2008). Conversely, Programmed Death ligand-1 (PDL-1) mediated inhibition limits the expansion of Treg cells by controlling STAT-5 phosphorylation (pSTAT-5) (Franceschini et al., 2009), which can diminish suppressive function of Tregs, lead to viral load control and ultimately ensure long-lasting survival of the host.
HCV is able to induce the up-regulation of different negative co-stimulatory molecules in order to provoke an anergic status on HCV-specific T cells. Expression of the inhibitory receptor PD-1 is one of these molecules involved in the generation of a state of exhaustion on HCV-specific CD8+ T cells during chronic HCV infection (Barber et al., 2006; Larrubia et al., 2009b). Importance of expression of PD-1 in HCV-specific T cell failure mechanism has been observed (Golden-Mason et al., 2007; Radziewicz et al., 2007), which can hinder by mutation in T cell epitopes (Rutebemberwa et al., 2008). In addition, blocking of PD-1 signaling resulted in the functional restoration of blood-derived HCV-specific CD8+ T cell responses in chronic infection (Penna et al., 2007; Radziewicz et al., 2007). However, the PD-1 alone is not sufficient in defining exhausted HCV-specific CD8+ T cells during HCV infection. To restore function of HCV-specific T cells isolated from liver biopsies of infected patients, there is need of CTLA4 blockade in addition to PD-1 blockade (Nakamoto et al., 2009). In addition, the co-expression of other inhibitory receptors such as 2B4, CD160, Tim-3 and KLRG1 occurred in about half of HCV-specific CD8+ T cell responses and correlate with low or intermediate level of CD127 expression, impaired proliferative capacity, an intermediate T cell differentiation stage (Bengsch et al., 2010). These data indicates that HCV infection modulates different negative co-stimulatory molecules to favor the development of HCV-specific CD8+ T cell exhaustion. On the other hand, HCV infection is also able to regulate pro-apototic pathways to induce HCV-specific T cell deletion in order to escape from immune response. HCV-specific CTLs from chronic patients targeting the virus express an exhausted phenotype associated to the up-regulation of the pro-apototic...
molecule Bim. The activity of this molecule is contra-regulated by the anti-apoptotic molecule Mcl-1. Interestingly, the reactivity of these cells is impaired but can be restored by blocking apoptotic pathways (Larrubia et al., 2011).

In summary, HCV is able to impair adaptive immune response at different levels. The effector population in charged of HCV clearing is defective because HCV is able to induce on those cells anergy and apoptosis (Fig.-5). Moreover, HCV is able to escape humoral response and cellular response by escape mutations in immunodominant epitopes. Finally, HCV is also quite efficient in the impairment of the specific T helper response, which is essential to organize the humoral and cellular response. To perform all these immune-escape strategies, HCV takes advantage of the pro-anergenic environment of the infected liver, because HCV-specific T cell priming at this level is not efficient to develop adequate effector cells. As it was commented for HBV infection, HCV immune response restoration could be an interesting therapeutic tool to help in viral clearance in chronic patients.

4. Accepted model of HBV&HCV pathogenesis

As previously commented, specific CTLs play a central role in HBV&HCV immunopathogenesis. These cells are able to kill some infected hepatocytes inducing a minor liver damage, but also they secrete type-I cytokines responsible for non-cytopathic virus clearing. To attract these cells into the liver the infected hepatocytes secrete another kind of cytokines called chemokines. The migration of lymphocytes to the liver is a complex process including adhesion, rolling, triggering, and transendothelial migration. Chemokines and their receptors play an essential role in this multistep pathway (Springer, 1994). During primoinfection, when the adaptive immune system is not able to control infection, the infected hepatocytes continue secreting chemokines to try to attract more defensive cells. In viral chronic hepatitis, the expression of different chemokines in the liver has been described. CXCL-10 is increased in the liver and peripheral blood during viral chronic hepatitis (Larrubia et al., 2007; Shields et al., 1999; Tan et al., 2010; Wang et al., 2008). This molecule is produced by hepatocytes and sinusoidal endothelial cells. Moreover, CXCL9 and CXCL11 are also increased in serum and liver of subjects with chronic viral hepatitis (Bieche et al., 2005). CXCL9 is detected primarily on sinusoidal endothelial cells, while CXCL-11 is produced mainly by hepatocytes (Apolinario et al., 2002). CCL5 intrahepatic expression is also elevated in viral chronic hepatitis and is produced by hepatocytes, sinusoidal endothelial cells and biliary epithelium. Finally, several studies have reported an increased level of CCL3 and CCL4 either in the liver or in serum. These molecules are detected on endothelial cells, on some hepatocytes and biliary epithelial cells (Apolinario et al., 2004). The expression of all these chemokines in the liver can be induced directly by viral proteins. Previous reports have shown a high hepatocyte synthesis of CXCL10, CXCL9 and CCL5, induced by some HCV proteins such as NS5A and core (Apolinario et al., 2005), although a recent in-vitro study suggests that HCV proteins could also decrease CCL5 and CXCL10 genes expression (Sillanpaa et al., 2008). All these chemokines recruit T cells with a Th1/Tc1 phenotype, expressing specific chemokine receptors such as CCR5 and CXCR3. The non-ELR-CXC chemokine attracts CXCR3 expressing T cells while CC chemokine attract CCR5 expressing T cells to the liver. Consequently, in viral chronic hepatitis, an intrahepatic enrichment of CCR5 and CXCR3 expressing T cells, located in hepatic lobule and portal tracts has been shown, while these populations are very infrequent in uninfected subjects (Bertoletti & Maini, 2000; Larrubia et al., 2008) (Fig.- 6).
Persistent HBV&HCV infection is characterized by a non-specific inflammatory infiltrate in the liver, mainly of CD8+ cells (Sprengers et al., 2005), responsible for liver damage. These cells are attracted by the interaction between the intrahepatic secreted chemokines and the chemokine receptors expressed on T cells. Actually, previous reports have shown a correlation between liver inflammation and liver infiltrating CXCR3/CCR5 expressing T cells. The frequency of these cells was positively correlated with portal and lobular inflammation but not with liver fibrosis (Larrubia et al., 2007). These data suggest that CCR5 and CXCR3 could play an important role in chronic liver damage by means of inflammatory T cells recruitment into the liver. Moreover, several previous studies have also shown a correlation between liver inflammation and chemokine levels. Intrahepatic CXCL10 mRNA levels are associated with intralobular inflammation (Harvey et al., 2003). Similarly, CXCL9 and CXCL11 correlate with the grade of liver inflammation (Helbig et al., 2004). Furthermore, CC chemokines are also correlated with the intrahepatic inflammatory activity (Kusano et al., 2000). Clearly, intrahepatic CCL5 positive cells correlate with the inflammatory activity. Bearing in mind all the previous data it is possible to speculate that

![Scheme showing the role of T cell intrahepatic recruitment according to the degree of liver damage and viral control.](https://www.intechopen.com)

Fig. 6. Scheme showing the role of T cell intrahepatic recruitment according to the degree of liver damage and viral control. In resolved HBV/HCV infection an adequate effector T cell response is attracted to the liver to clear the virus. After that, a memory T cell population is continuously patrolling the liver to keep under control viral traces. Nevertheless, in persistent infection after specific T cells failure to control infection, a non-specific inflammatory infiltrate is sequestered into the liver, responsible of the persistent liver damage.
chemokines are secreted in the infected liver to attract an adaptive immune response able to clear the virus. Unfortunately, when the specific response fails these chemokines also attract non-specific mononuclear and polymorphonuclear cells, which are not able to remove the virus but produce liver inflammation (Kakimi et al., 2001). Therefore, as chemokines are nonspecific chemoattractants, intrahepatic inflammatory infiltrate during chronic infection is mainly non-virus-specific and consequently unable to eliminate the infection, but able to produce cytokines capable of initiating and perpetuating hepatic fibrogenesis (Bertoletti & Maini, 2000; Bertoletti et al., 2010; Friedman, 2003; Larrubia et al., 2008).

5. Mechanisms to restore adaptive immune response in HBV&HCV infection

Bearing in mind that specific T responses are essential to control HCV during natural immune response, several studies have been performed to analyze the role of different therapeutic approaches on T cell response to know whether it is possible to reverse T cell dysfunction in-vivo. Longitudinal analysis of HBV-specific responses during IFN-α treatment did not show a significant increase of these responses during treatment (Sprengers et al., 2007). Nevertheless, it was observed an improvement after treatment in patients with resolved infection (Carotenuto et al., 2009). During HCV infection, the same scenario was observed (Barnes et al., 2002), although some studies have demonstrated a restoration of T cell response in sustained viral responders (Kamal et al., 2002). However, patients presenting a better HCV-specific CD8 cell proliferative potential at baseline, are more likely to present a rapid and sustained viral response. Moreover, after treatment a HCV-specific T-cell response enhancement is observed in sustained viral responders (Pilli et al., 2007). The absence of T cell reactivity improvement during treatment could be due to the direct anti-proliferative effect of IFN-α. Obviously, this effect could counteract the positive consequence of decreasing viral pressure on specific-T cells during treatment. Nevertheless, these data also could suggest that is important to restore a specific T cell response, at least at the end of treatment, to keep under control residual viral traces. In HBV infection, several papers dealing with the role of nucleot(s)ide analogues (NUCs) treatment on anti-HBV immune response have shown that they are able to reconstitute temporally HBV-specific CD4 and CD8 responses (Boni et al., 1998; Cooksley et al., 2008). Moreover, these treatments can decrease the frequency of Tregs during treatment (Stoop et al., 2007) and specifically to decrease the ratio Treg:Th17 (Zhang et al., 2010). These data suggest that the NUCs are controlling the infection not only through an anti-viral effect but also helping to restore specific immune response. In any case, all these effects on specific T cells are partial and limited in time. For that reason other immunoregulatory therapeutic approaches are being considered. Several pre-clinical studies have been performed to try to restore HBV/HCV specific responses in-vitro and in animal models. Modulation of negative co-stimulatory molecules, in addition to blocking immunosuppressive cytokines could be promising strategies to restore an effective T cell response. The modulation of negative co-stimulatory molecules, such as PD-1, CTLA-4, Tim-3, has shown in-vitro to increase specific-T cell reactivity. This can be also enhanced using Abs to block the regulatory cytokine IL-10. Experts in immunotherapy have suggested that after restoring a T cell response could be necessary to boost that response using a therapeutic vaccine. Although these results seem to be quite promising, the blockade of negative co-stimulatory pathways in addition to IL-10 could lead to the development of autoimmune diseases, which could prevent the use of this strategy as a therapeutic tool in humans. Therefore, more research is necessary in this field.
before these strategies are suitable for the treatment of chronic viral infections (Ferrari, 2008; Fisicaro et al., 2010; Larrubia et al., 2011; Nakamoto et al., 2009).

6. Conclusions

HBV & HCV are two hepatotropic non-cytopathic viruses able to develop a chronic liver disease. The innate immune response is defective in both infections, residing the viral control in the efficacy of adaptive immune response. HBV&HCV specific CTL response play a central role in viral control through cytopathic and non-cytopathic mechanisms. Nevertheless, during persistent infection, adaptive response is impaired due to exhaustion and deletion. Several in-vitro strategies have shown to be effective in its restoration but it is necessary more research before these approaches can be applied to clinical practice. Finally, when the virus is not controlled by adaptive response a non-specific inflammatory infiltrate is attracted to the liver which is responsible for the persistent low-grade liver damage, allowing the generation of liver fibrosis during disease progression.

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8. References


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There are a lot of important issues related to viral hepatitis studies: molecular biology of viruses, laboratory diagnostics, epidemiology, treatment etc. However, there is a number of special textbooks and monographs on the subject. Considering this fact and rather fast progress in our understanding of the problem this book focuses on the important sections of the problem immune pathogenesis of parenterally transmitted viral hepatitis and some aspects of hepatitis diagnostics. Seven chapters were prepared by several groups of researchers to share information and results of studies with specialists working in the field and persons who are interested to learn about the viral hepatitis issue. The Nobel Prize Committee (the field of physiology and medicine, 2011) awarded Bruce A. Beutler and Jules A. Hoffmann for their discoveries concerning the activation of innate immunity whilst Ralph M. Steinman was awarded for his discovery of the dendritic cell and its role in adaptive immunity. We are proud to say that our book is in line with these discoveries, because 3 chapters cover the problems of innate and adaptive immune response in case of viral hepatitis.

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