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).

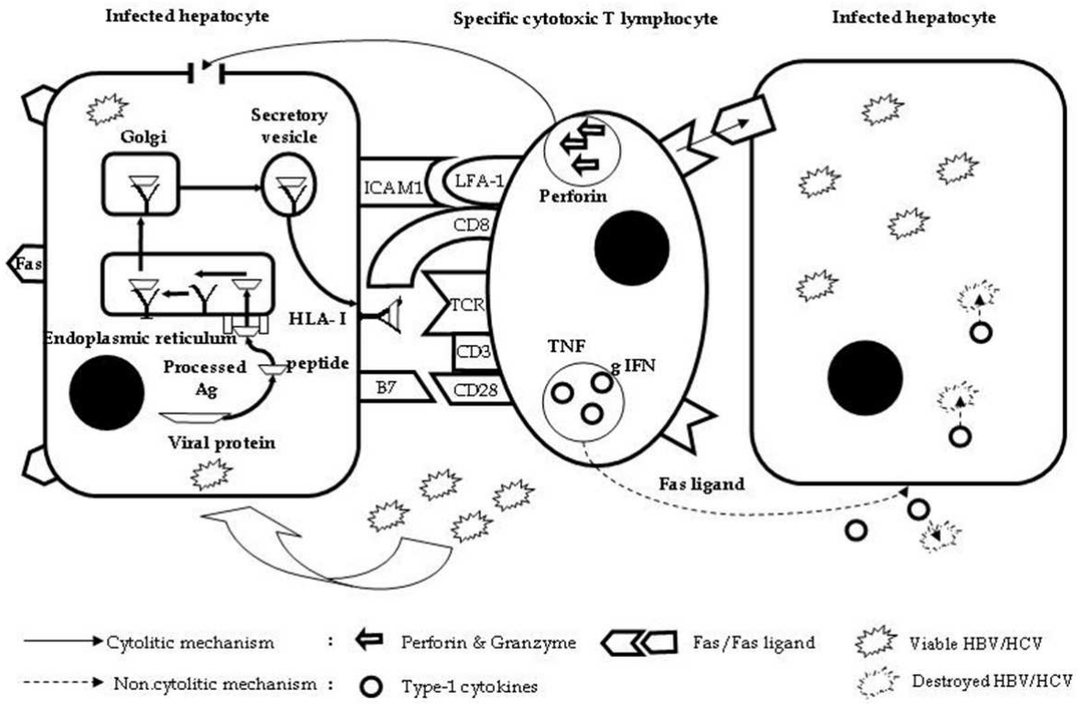


Fig. 1. Cytolitic and non-cytolitic mechanisms to destroy hepatotropic viruses by specific cytotoxic T cells

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.

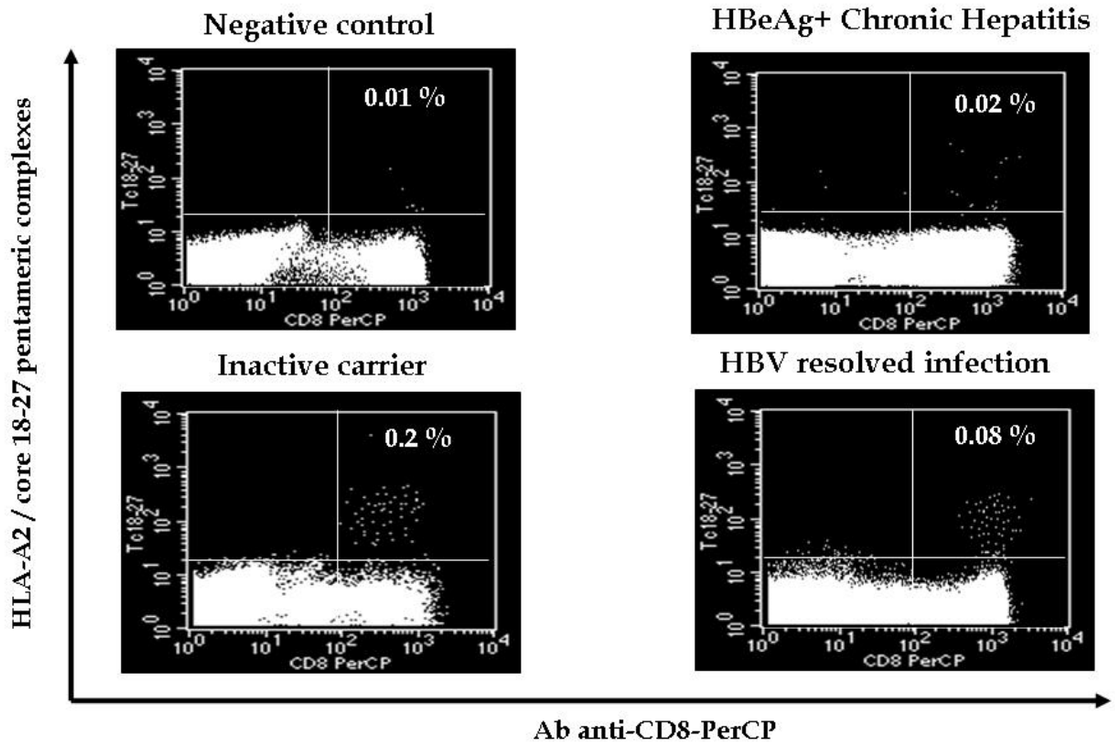


Fig. 2. FACS® dot-plots from peripheral blood mononuclear cells of HBV infected patients with different HBV control stained directly ex-vivo with Ab against CD8 plus multimeric HLA-A2/core 18-27 complexes. A negative correlation between viral control and frequency of HBV-sepcific CD8+ cells is observed. 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).

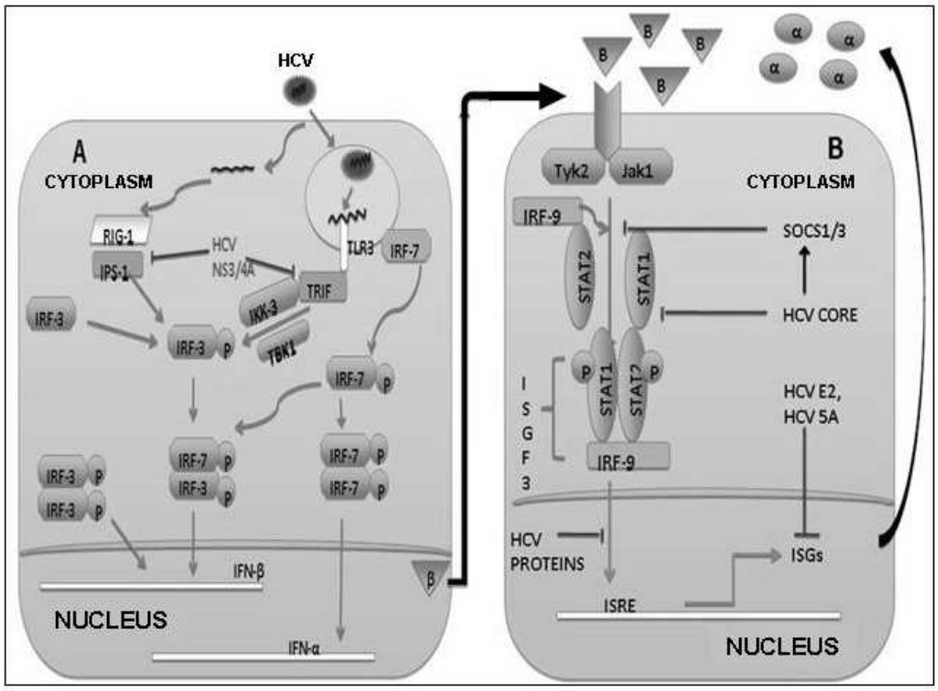


Fig. 3. Evasion of Innate immune response by HCV: (A) Interference in IFN synthesis: Blocking of TLR 3 and RIG-1 signalling respectively, by cleavage of the adaptor molecule TRIF and IPS-1 via HCV NS3/4A; (B) Interference in IFN-induced effector mechanisms: Binding of IFN β and its receptor with TYK2 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.

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 down-stream 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; Thimme *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 evident 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; Amarapurkar & Amarapurkar, 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).

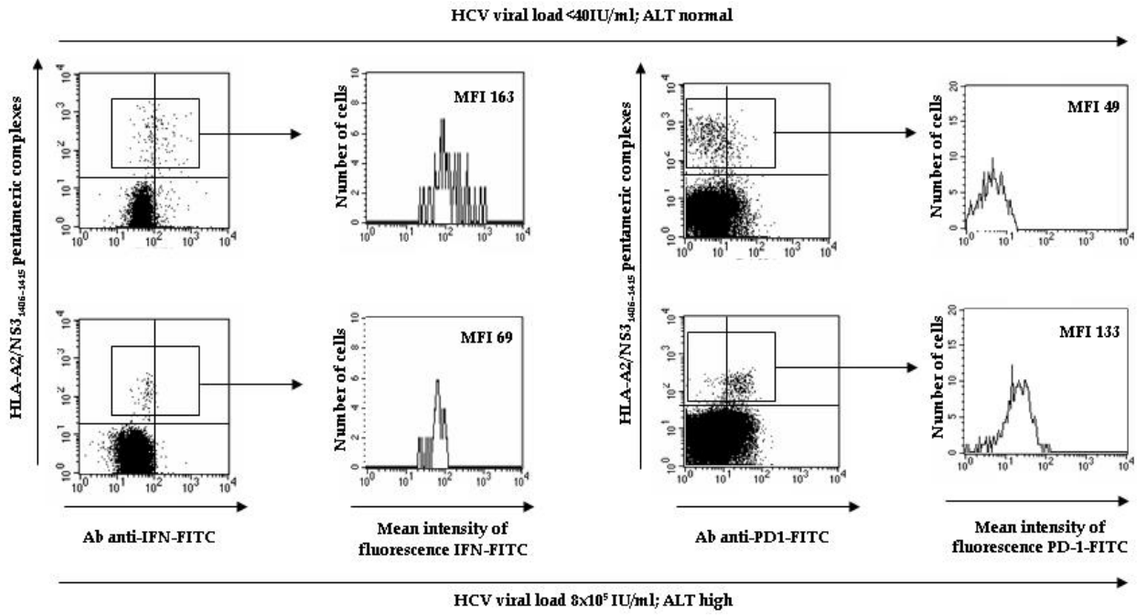


Fig. 4. FACS® dot-plots and histogramas of CD8+ cells from HCV patients with different viral control. CD8+ cells were stained with Abs anti IFN-gamma and anti-PD-1 plus pentemeric HLA-A2/NS3-1406 peptide complexes. A negative correlation between PD-1 expression and IFN-gamma production by HCV-specific T cells according to viral control is shown.

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 auto-immunity 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 Tregs 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.

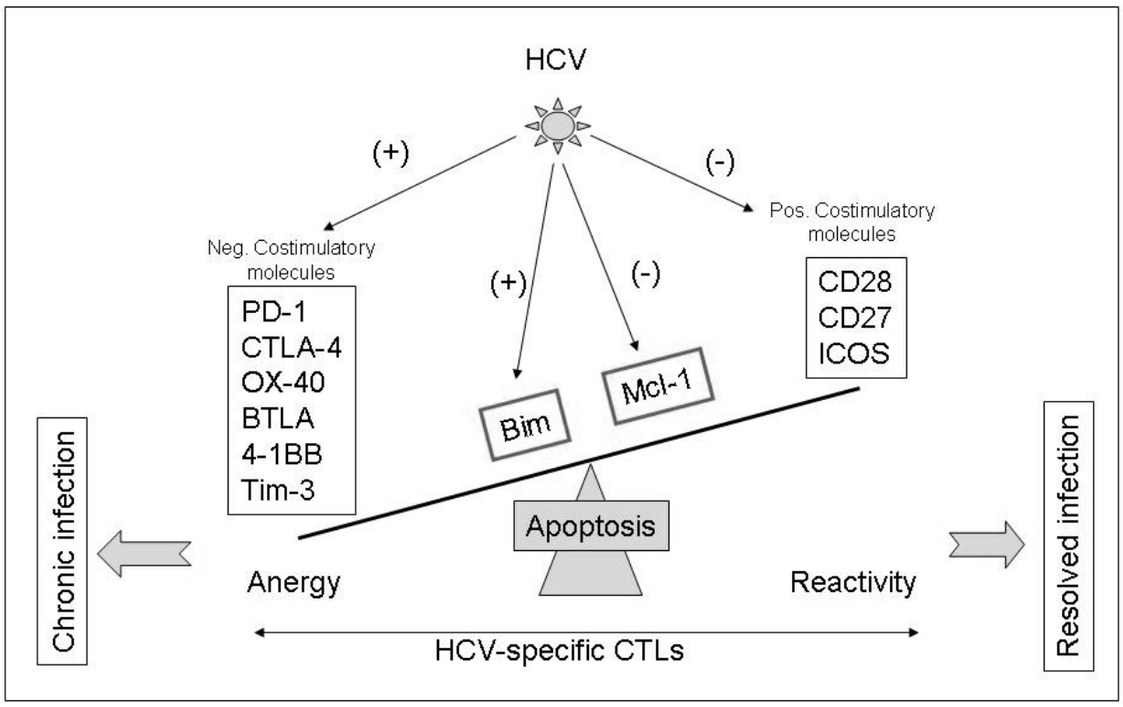


Fig. 5. Scheme showing the balance between co-stimulatory/apoptotic molecules and HCV-specific CTLs reactivity according to infection outcome. Neg.: negative. Pos.: positive. CTLs: cytotoxic T lymphocytes. HCV: hepatitis C virus. (+): possible molecules induced by HCV infection. (-): possible molecules down-regulated by HCV infection.

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

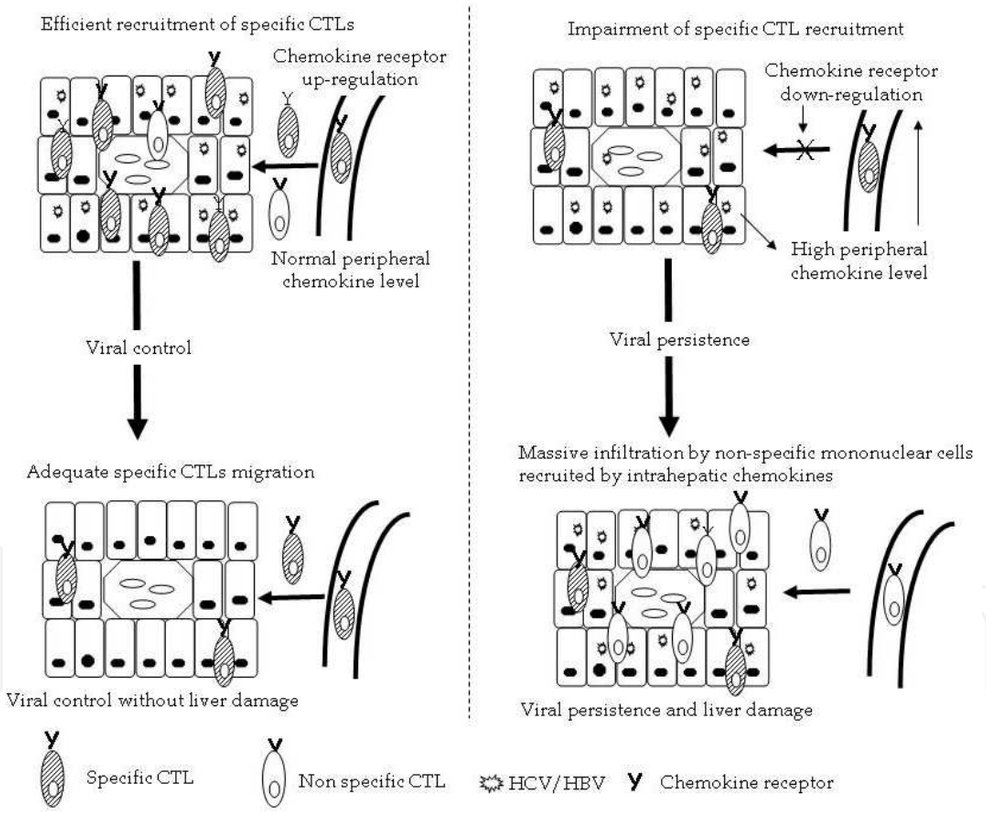


Fig. 6. Scheme showing the role of T cell intrahepatic recruitment according to the degree of liver damage and viral control. In resolved HBV/HCV infection an adequate effector T cell response is attracted to the liver to clear the virus. After that, a memory T cell population is continuously patrolling the liver to keep under control viral traces. Nevertheless, in persistent infection after specific T cells failure to control infection, a non-specific immflamatory 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 immunosupressive 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.

7. Acknowledgments

This book chapter was supported by grants from "Fiscam" (PI-2007/32), (PI-2010/022) and "Fundación de Investigación Médica Mutua Madrileña" (2548/2008), Spain. Benito S and Lokhande MU were supported by research grants from "Fiscam" (MOV-2007_JI/18) and, "Asociación de Hepatología Translacional" (AHT10/01) Spain, respectively.

8. References

Abel, M., Sene, D., Pol, S., Bourliere, M., Poynard, T., Charlotte, F., Cacoub, P. & Caillat-Zucman, S. (2006). Intrahepatic virus-specific IL-10-producing CD8 T cells prevent liver damage during chronic hepatitis C virus infection. *Hepatology* 44, 1607-1616.

Accapezzato, D., Francavilla, V., Paroli, M., Casciaro, M., Chircu, L. V., Cividini, A., Abrignani, S., Mondelli, M. U. & Barnaba, V. (2004). Hepatic expansion of a virus-specific regulatory CD8(+) T cell population in chronic hepatitis C virus infection. *J Clin Invest* 113, 963-972.

Agnello, V., Abel, G., Elfahal, M., Knight, G. B. & Zhang, Q. X. (1999). Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 96, 12766-12771.

Agnello, V. & De Rosa, F. G. (2004). Extrahepatic disease manifestations of HCV infection: some current issues. *J Hepatol* 40, 341-352.

Ahlenstiel, G., Titerence, R. H., Koh, C., Edlich, B., Feld, J. J., Rotman, Y., Ghany, M. G., Hoofnagle, J. H., Liang, T. J., Heller, T. & Rehermann, B. (2010). Natural killer cells are polarized toward cytotoxicity in chronic hepatitis C in an interferon-alfa-dependent manner. *Gastroenterology* 138, 325-335.

Alatrakchi, N., Graham, C. S., van der Vliet, H. J., Sherman, K. E., Exley, M. A. & Koziel, M. J. (2007). Hepatitis C virus (HCV)-specific CD8+ cells produce transforming growth factor beta that can suppress HCV-specific T-cell responses. *J Virol* 81, 5882-5892.

Albert, M. L., Decalf, J. & Pol, S. (2008). Plasmacytoid dendritic cells move down on the list of suspects: in search of the immune pathogenesis of chronic hepatitis C. *J Hepatol* 49, 1069-1078.

Alexopoulou, L., Holt, A. C., Medzhitov, R. & Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413, 732-738.

Alter, H. J., Purcell, R. H., Holland, P. V. & Popper, H. (1978). Transmissible agent in non-A, non-B hepatitis. *Lancet* 1, 459-463.

Amarapurkar, D. N. & Amarapurkar, A. D. (2002). Extrahepatic manifestations of viral hepatitis. *Ann Hepatol* 1, 192-195.

Anthony, D. D., Yonkers, N. L., Post, A. B., Asaad, R., Heinzel, F. P., Lederman, M. M., Lehmann, P. V. & Valdez, H. (2004). Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. *J Immunol* 172, 4907-4916.

Apolinario, A., Diago, M., Lo Iacono, O., Lorente, R., Perez, C., Majano, P. L., Clemente, G. & Garcia-Monzon, C. (2004). Increased circulating and intrahepatic T-cell-specific chemokines in chronic hepatitis C: relationship with the type of virological response to peginterferon plus ribavirin combination therapy. *Aliment Pharmacol Ther* 19, 551-562.

Apolinario, A., Majano, P. L., Alvarez-Perez, E., Saez, A., Lozano, C., Vargas, J. & Garcia-Monzon, C. (2002). Increased expression of T cell chemokines and their receptors in chronic hepatitis C: relationship with the histological activity of liver disease. *Am J Gastroenterol* 97, 2861-2870.

Apolinario, A., Majano, P. L., Lorente, R., Nunez, O., Clemente, G. & Garcia-Monzon, C. (2005). Gene expression profile of T-cell-specific chemokines in human hepatocyte-derived cells: evidence for a synergistic inducer effect of cytokines and hepatitis C virus proteins. *J Viral Hepat* 12, 27-37.

Appay, V., van Lier, R. A., Sallusto, F. & Roederer, M. (2008). Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A* 73, 975-983.

Barber, D. L., Wherry, E. J., Masopust, D., Zhu, B., Allison, J. P., Sharpe, A. H., Freeman, G. J. & Ahmed, R. (2006). Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439, 682-687.

Barnes, E., Harcourt, G., Brown, D., Lucas, M., Phillips, R., Dusheiko, G. & Klenerman, P. (2002). The dynamics of T-lymphocyte responses during combination therapy for chronic hepatitis C virus infection. *Hepatology* 36, 743-754.

Barton, G. M. (2007). Viral recognition by Toll-like receptors. *Semin Immunol* 19, 33-40.

Bartosch, B., Dubuisson, J. & Cosset, F. L. (2003a). Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. *J Exp Med* 197, 633-642.

Bartosch, B., Vitelli, A., Granier, C., Goujon, C., Dubuisson, J., Pascale, S., Scarselli, E., Cortese, R., Nicosia, A. & Cosset, F. L. (2003b). Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. *J Biol Chem* 278, 41624-41630.

Bassett, S. E., Thomas, D. L., Brasky, K. M. & Lanford, R. E. (1999). Viral persistence, antibody to E1 and E2, and hypervariable region 1 sequence stability in hepatitis C virus-inoculated chimpanzees. *J Virol* 73, 1118-1126.

Bauer, S., Kirschning, C. J., Hacker, H., Redecke, V., Hausmann, S., Akira, S., Wagner, H. & Lipford, G. B. (2001). Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci U S A* 98, 9237-9242.

Baumert, T. F., Ito, S., Wong, D. T. & Liang, T. J. (1998). Hepatitis C virus structural proteins assemble into viruslike particles in insect cells. *J Virol* 72, 3827-3836.

Benedicto, I., Molina-Jimenez, F., Bartosch, B., Cosset, F. L., Lavillette, D., Prieto, J., Moreno-Otero, R., Valenzuela-Fernandez, A., Aldabe, R., Lopez-Cabrera, M. & Majano, P. L. (2009). The tight junction-associated protein occludin is required for a postbinding step in hepatitis C virus entry and infection. *J Virol* 83, 8012-8020.

Bengsch, B., Seigel, B., Ruhl, M., Timm, J., Kuntz, M., Blum, H. E., Pircher, H. & Thimme, R. (2010). Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. *PLoS Pathog* 6, e1000947.

Bertoletti, A. & Maini, M. K. (2000). Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection?. *Curr Opin Microbiol* 3, 387-392.

Bertoletti, A., Maini, M. K. & Ferrari, C. (2010). The host-pathogen interaction during HBV infection: immunological controversies. *Antivir Ther* 15 Suppl 3, 15-24.

Bieche, I., Asselah, T., Laurendeau, I., Vidaud, D., Degot, C., Paradis, V., Bedossa, P., Valla, D. C., Marcellin, P. & Vidaud, M. (2005). Molecular profiling of early stage liver fibrosis in patients with chronic hepatitis C virus infection. *Virology* 332, 130-144.

Biron, C. A., Nguyen, K. B., Pien, G. C., Cousens, L. P. & Salazar-Mather, T. P. (1999). Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 17, 189-220.

Blanchard, E., Belouzard, S., Goueslain, L., Wakita, T., Dubuisson, J., Wychowski, C. & Rouille, Y. (2006). Hepatitis C virus entry depends on clathrin-mediated endocytosis. *J Virol* 80, 6964-6972.

Blight, K. J., Kolykhalov, A. A. & Rice, C. M. (2000). Efficient initiation of HCV RNA replication in cell culture. *Science* 290, 1972-1974.

Blight, K. J., McKeating, J. A., Marcotrigiano, J. & Rice, C. M. (2003). Efficient replication of hepatitis C virus genotype 1a RNAs in cell culture. *J Virol* 77, 3181-3190.

Blight, K. J., McKeating, J. A. & Rice, C. M. (2002). Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication. *J Virol* 76, 13001-13014.

Bluestone, J. A. & Abbas, A. K. (2003). Natural versus adaptive regulatory T cells. *Nat Rev Immunol* 3, 253-257.

Bode, J. G., Ludwig, S., Ehrhardt, C., Albrecht, U., Erhardt, A., Schaper, F., Heinrich, P. C. & Haussinger, D. (2003). IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. *FASEB J* 17, 488-490.

Boettler, T., Spangenberg, H. C., Neumann-Haefelin, C., Panther, E., Urbani, S., Ferrari, C., Blum, H. E., von Weizsacker, F. & Thimme, R. (2005). T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. *J Virol* 79, 7860-7867.

Boni, C., Bertoletti, A., Penna, A., Cavalli, A., Pilli, M., Urbani, S., Scognamiglio, P., Boehme, R., Panebianco, R., Fiaccadori, F. & Ferrari, C. (1998). Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. *J Clin Invest* 102, 968-975.

Boni, C., Fisicaro, P., Valdatta, C., Amadei, B., Di Vincenzo, P., Giuberti, T., Laccabue, D., Zerbini, A., Cavalli, A., Missale, G., Bertoletti, A. & Ferrari, C. (2007). Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. *J Virol* 81, 4215-4225.

Bowen, D. G., Shoukry, N. H., Grakoui, A., Fuller, M. J., Cawthon, A. G., Dong, C., Hasselschwert, D. L., Brasky, K. M., Freeman, G. J., Seth, N. P., Wucherpfennig, K. W., Houghton, M. & Walker, C. M. (2008). Variable patterns of programmed death-1 expression on fully functional memory T cells after spontaneous resolution of hepatitis C virus infection. *J Virol* 82, 5109-5114.

Bowen, D. G. & Walker, C. M. (2005). Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature* 436, 946-952.

Bukh, J. (2004). A critical role for the chimpanzee model in the study of hepatitis C. *Hepatology* 39, 1469-1475.

Cabrera, R., Tu, Z., Xu, Y., Firpi, R. J., Rosen, H. R., Liu, C. & Nelson, D. R. (2004). An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 40, 1062-1071.

Carotenuto, P., Artsen, A., Niesters, H. G., Osterhaus, A. D. & Pontesilli, O. (2009). In vitro use of autologous dendritic cells improves detection of T cell responses to hepatitis B virus (HBV) antigens. *J Med Virol* 81, 332-339.

Cooksley, H., Chokshi, S., Maayan, Y., Wedemeyer, H., Andreone, P., Gilson, R., Warnes, T., Paganin, S., Zoulim, F., Frederick, D., Neumann, A. U., Brosgart, C. L. & Naoumov, N. V. (2008). Hepatitis B virus e antigen loss during adefovir dipivoxil therapy is associated with enhanced virus-specific CD4+ T-cell reactivity. *Antimicrob Agents Chemother* 52, 312-320.

Chang, K. M., Rehermann, B., McHutchison, J. G., Pasquinelli, C., Southwood, S., Sette, A. & Chisari, F. V. (1997). Immunological significance of cytotoxic T lymphocyte epitope variants in patients chronically infected by the hepatitis C virus. *J Clin Invest* 100, 2376-2385.

Chen, Z., Cheng, Y., Xu, Y., Liao, J., Zhang, X., Hu, Y., Zhang, Q., Wang, J., Zhang, Z., Shen, F. & Yuan, Z. (2008). Expression profiles and function of Toll-like receptors 2 and 4 in peripheral blood mononuclear cells of chronic hepatitis B patients. *Clin Immunol* 128, 400-408.

Choudhuri, K., Kearney, A., Bakker, T. R. & van der Merwe, P. A. (2005). Immunology: how do T cells recognize antigen? *Curr Biol* 15, R382-385.

Das, A., Hoare, M., Davies, N., Lopes, A. R., Dunn, C., Kennedy, P. T., Alexander, G., Finney, H., Lawson, A., Plunkett, F. J., Bertoletti, A., Akbar, A. N. & Maini, M. K. (2008). Functional skewing of the global CD8 T cell population in chronic hepatitis B virus infection. *J Exp Med* 205, 2111-2124.

Decalf, J., Fernandes, S., Longman, R., Ahloulay, M., Audat, F., Lefrerre, F., Rice, C. M., Pol, S. & Albert, M. L. (2007). Plasmacytoid dendritic cells initiate a complex chemokine and cytokine network and are a viable drug target in chronic HCV patients. *J Exp Med* 204, 2423-2437.

Diepolder, H. M., Zachoval, R., Hoffmann, R. M., Wierenga, E. A., Santantonio, T., Jung, M. C., Eichenlaub, D. & Pape, G. R. (1995). Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. *Lancet* 346, 1006-1007.

Dolganiuc, A., Chang, S., Kodys, K., Mandrekar, P., Bakis, G., Cormier, M. & Szabo, G. (2006). Hepatitis C virus (HCV) core protein-induced, monocyte-mediated mechanisms of reduced IFN-alpha and plasmacytoid dendritic cell loss in chronic HCV infection. *J Immunol* 177, 6758-6768.

Dunn, C., Brunetto, M., Reynolds, G., Christophides, T., Kennedy, P. T., Lampertico, P., Das, A., Lopes, A. R., Borrow, P., Williams, K., Humphreys, E., Afford, S., Adams, D. H., Bertoletti, A. & Maini, M. K. (2007). Cytokines induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. *J Exp Med* 204, 667-680.

Dunn, C., Peppa, D., Khanna, P., Nebbia, G., Jones, M., Brendish, N., Lascar, R. M., Brown, D., Gilson, R. J., Tedder, R. J., Dusheiko, G. M., Jacobs, M., Klenerman, P. & Maini, M. K. (2009). Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. *Gastroenterology* 137, 1289-1300.

Duramad, O., Fearon, K. L., Chan, J. H., Kanzler, H., Marshall, J. D., Coffman, R. L. & Barrat, F. J. (2003). IL-10 regulates plasmacytoid dendritic cell response to CpG-containing immunostimulatory sequences. *Blood* 102, 4487-4492.

Dustin, L. B. & Rice, C. M. (2007). Flying under the radar: the immunobiology of hepatitis C. *Annu Rev Immunol* 25, 71-99.

Evans, M. J., von Hahn, T., Tscherne, D. M., Syder, A. J., Panis, M., Wolk, B., Hatziioannou, T., McKeating, J. A., Bieniasz, P. D. & Rice, C. M. (2007). Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 446, 801-805.

Farci, P., Shimoda, A., Coiana, A., Diaz, G., Peddis, G., Melpolder, J. C., Strazzera, A., Chien, D. Y., Munoz, S. J., Balestrieri, A., Purcell, R. H. & Alter, H. J. (2000). The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* 288, 339-344.

Ferrari, C. (2008). Therapeutic vaccination for hepatitis C: can protective T-cell responses be restored after prolonged antigen exposure?. *Gastroenterology* 134, 1601-1604.

Ferrari, C., Missale, G., Boni, C. & Urbani, S. (2003). Immunopathogenesis of hepatitis B. *J Hepatol* 39 Suppl 1, S36-42.

Fisicaro, P., Valdatta, C., Boni, C., Massari, M., Mori, C., Zerbini, A., Orlandini, A., Sacchelli, L., Missale, G. & Ferrari, C. (2009). Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 58, 974-982.

Fisicaro, P., Valdatta, C., Massari, M., Loggi, E., Biasini, E., Sacchelli, L., Cavallo, M. C., Silini, E. M., Andreone, P., Missale, G. & Ferrari, C. (2010). Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology* 138, 682-693, 693 e681-684.

Fitzgerald-Bocarsly, P., Dai, J. & Singh, S. (2008). Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history. *Cytokine Growth Factor Rev* 19, 3-19.

Foy, E., Li, K., Wang, C., Sumpter, R., Jr., Ikeda, M., Lemon, S. M. & Gale, M., Jr. (2003). Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. *Science* 300, 1145-1148.

Francavilla, V., Accapezzato, D., De Salvo, M., Rawson, P., Cosimi, O., Lipp, M., Cerino, A., Cividini, A., Mondelli, M. U. & Barnaba, V. (2004). Subversion of effector CD8+ T cell differentiation in acute hepatitis C virus infection: exploring the immunological mechanisms. *Eur J Immunol* 34, 427-437.

Franceschini, D., Paroli, M., Francavilla, V., Videtta, M., Morrone, S., Labbadia, G., Cerino, A., Mondelli, M. U. & Barnaba, V. (2009). PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. *J Clin Invest* 119, 551-564.

Frelin, L., Wahlstrom, T., Tucker, A. E., Jones, J., Hughes, J., Lee, B. O., Billaud, J. N., Peters, C., Whitacre, D., Peterson, D. & Milich, D. R. (2009). A mechanism to explain the selection of the hepatitis e antigen-negative mutant during chronic hepatitis B virus infection. *J Virol* 83, 1379-1392.

Fried, M. W., Shiffman, M. L., Reddy, K. R., Smith, C., Marinos, G., Goncales, F. L., Jr., Haussinger, D., Diago, M., Carosi, G., Dhumeaux, D., Craxi, A., Lin, A., Hoffman, J. & Yu, J. (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347, 975-982.

Friedman, S. L. (2003). Liver fibrosis -- from bench to bedside. *J Hepatol* 38 Suppl 1, S38-53.

Furuichi, Y., Tokuyama, H., Ueha, S., Kurachi, M., Moriyasu, F. & Kakimi, K. (2005). Depletion of CD25+CD4+T cells (Tregs) enhances the HBV-specific CD8+ T cell response primed by DNA immunization. *World J Gastroenterol* 11, 3772-3777.

Gal-Tanamy, M., Keck, Z. Y., Yi, M., McKeating, J. A., Patel, A. H., Foung, S. K. & Lemon, S. M. (2008). In vitro selection of a neutralization-resistant hepatitis C virus escape mutant. *Proc Natl Acad Sci U S A* 105, 19450-19455.

Gale, M., Jr. & Foy, E. M. (2005). Evasion of intracellular host defence by hepatitis C virus. *Nature* 436, 939-945.

Garry, R. F. & Dash, S. (2003). Proteomics computational analyses suggest that hepatitis C virus E1 and pestivirus E2 envelope glycoproteins are truncated class II fusion proteins. *Virology* 307, 255-265.

Gealy, C., Denson, M., Humphreys, C., McSharry, B., Wilkinson, G. & Caswell, R. (2005). Posttranscriptional suppression of interleukin-6 production by human cytomegalovirus. *J Virol* 79, 472-485.

Gerlach, J. T., Diepolder, H. M., Jung, M. C., Gruener, N. H., Schraut, W. W., Zachoval, R., Hoffmann, R., Schirren, C. A., Santantonio, T. & Pape, G. R. (1999). Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. *Gastroenterology* 117, 933-941.

Golden-Mason, L., Madrigal-Estebas, L., McGrath, E., Conroy, M. J., Ryan, E. J., Hegarty, J. E., O'Farrelly, C. & Doherty, D. G. (2008). Altered natural killer cell subset distributions in resolved and persistent hepatitis C virus infection following single source exposure. *Gut* 57, 1121-1128.

Golden-Mason, L., Palmer, B., Klarquist, J., Mengshol, J. A., Castelblanco, N. & Rosen, H. R. (2007). Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. *J Virol* 81, 9249-9258.

Golden-Mason, L. & Rosen, H. R. (2006). Natural killer cells: primary target for hepatitis C virus immune evasion strategies? *Liver Transpl* 12, 363-372.

Grove, J., Huby, T., Stamataki, Z., Vanwolleghem, T., Meuleman, P., Farquhar, M., Schwarz, A., Moreau, M., Owen, J. S., Leroux-Roels, G., Balfe, P. & McKeating, J. A. (2007). Scavenger receptor BI and BII expression levels modulate hepatitis C virus infectivity. *J Virol* 81, 3162-3169.

Grove, J., Nielsen, S., Zhong, J., Bassendine, M. F., Drummer, H. E., Balfe, P. & McKeating, J. A. (2008). Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies. *J Virol* 82, 12020-12029.

Guidotti, L. G. & Chisari, F. V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 19, 65-91.

Guidotti, L. G. & Chisari, F. V. (2006). Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol* 1, 23-61.

Guidotti, L. G., Rochford, R., Chung, J., Shapiro, M., Purcell, R. & Chisari, F. V. (1999). Viral clearance without destruction of infected cells during acute HBV infection. *Science* 284, 825-829.

Guy, C. S., Mulrooney-Cousins, P. M., Churchill, N. D. & Michalak, T. I. (2008). Intrahepatic expression of genes affiliated with innate and adaptive immune responses immediately after invasion and during acute infection with woodchuck hepadnavirus. *J Virol* 82, 8579-8591.

Haberstroh, A., Schnober, E. K., Zeisel, M. B., Carolla, P., Barth, H., Blum, H. E., Cosset, F. L., Koutsoudakis, G., Bartenschlager, R., Union, A., Depla, E., Owsianka, A., Patel, A. H., Schuster, C., Stoll-Keller, F., Doffoel, M., Dreux, M. & Baumert, T. F. (2008). Neutralizing host responses in hepatitis C virus infection target viral entry at postbinding steps and membrane fusion. *Gastroenterology* 135, 1719-1728 e1711.

Haid, S., Pietschmann, T. & Pecheur, E. I. (2009). Low pH-dependent hepatitis C virus membrane fusion depends on E2 integrity, target lipid composition, and density of virus particles. *J Biol Chem* 284, 17657-17667.

Harvey, C. E., Post, J. J., Palladinetti, P., Freeman, A. J., Ffrench, R. A., Kumar, R. K., Marinos, G. & Lloyd, A. R. (2003). Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. *J Leukoc Biol* 74, 360-369.

Heim, M. H., Moradpour, D. & Blum, H. E. (1999). Expression of hepatitis C virus proteins inhibits signal transduction through the Jak-STAT pathway. *J Virol* 73, 8469-8475.

Helbig, K. J., Ruszkiewicz, A., Semendric, L., Harley, H. A., McColl, S. R. & Beard, M. R. (2004). Expression of the CXCR3 ligand I-TAC by hepatocytes in chronic hepatitis C and its correlation with hepatic inflammation. *Hepatology* 39, 1220-1229.

Hiroishi, K., Eguchi, J., Ishii, S., Hiraide, A., Sakaki, M., Doi, H., Omori, R. & Imawari, M. (2010). Immune response of cytotoxic T lymphocytes and possibility of vaccine development for hepatitis C virus infection. *J Biomed Biotechnol* 2010, 263810.

Honda, K., Yanai, H., Mizutani, T., Negishi, H., Shimada, N., Suzuki, N., Ohba, Y., Takaoka, A., Yeh, W. C. & Taniguchi, T. (2004). Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling. *Proc Natl Acad Sci U S A* 101, 15416-15421.

Hosel, M., Quasdorff, M., Wiegmann, K., Webb, D., Zedler, U., Broxtermann, M., Tedjokusumo, R., Esser, K., Arzberger, S., Kirschning, C. J., Langenkamp, A., Falk, C., Buning, H., Rose-John, S. & Protzer, U. (2009). Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* 50, 1773-1782.

Hoshino, K., Sugiyama, T., Matsumoto, M., Tanaka, T., Saito, M., Hemmi, H., Ohara, O., Akira, S. & Kaisho, T. (2006). IkappaB kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. *Nature* 440, 949-953.

Hsu, M., Zhang, J., Flint, M., Logvinoff, C., Cheng-Mayer, C., Rice, C. M. & McKeating, J. A. (2003). Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. *Proc Natl Acad Sci U S A* 100, 7271-7276.

Ikeda, M., Yi, M., Li, K. & Lemon, S. M. (2002). Selectable subgenomic and genome-length dicistronic RNAs derived from an infectious molecular clone of the HCV-N strain of hepatitis C virus replicate efficiently in cultured Huh7 cells. *J Virol* 76, 2997-3006.

Izaguirre, A., Barnes, B. J., Amrute, S., Yeow, W. S., Megjugorac, N., Dai, J., Feng, D., Chung, E., Pitha, P. M. & Fitzgerald-Bocarsly, P. (2003). Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. *J Leukoc Biol* 74, 1125-1138.

Kakimi, K., Guidotti, L. G., Koezuka, Y. & Chisari, F. V. (2000). Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med* 192, 921-930.

Kakimi, K., Lane, T. E., Wieland, S., Asensio, V. C., Campbell, I. L., Chisari, F. V. & Guidotti, L. G. (2001). Blocking chemokine responsive to gamma-2/interferon (IFN)-gamma inducible protein and monokine induced by IFN-gamma activity in vivo reduces the pathogenetic but not the antiviral potential of hepatitis B virus-specific cytotoxic T lymphocytes. *J Exp Med* 194, 1755-1766.

Kamal, S. M., Fehr, J., Roesler, B., Peters, T. & Rasenack, J. W. (2002). Peginterferon alone or with ribavirin enhances HCV-specific CD4 T-helper 1 responses in patients with chronic hepatitis C. *Gastroenterology* 123, 1070-1083.

Kann, M., Schmitz, A. & Rabe, B. (2007). Intracellular transport of hepatitis B virus. *World J Gastroenterol* 13, 39-47.

Kanto, T., Hayashi, N., Takehara, T., Tatsumi, T., Kuzushita, N., Ito, A., Sasaki, Y., Kasahara, A. & Hori, M. (1999). Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. *J Immunol* 162, 5584-5591.

Kapadia, S. B., Barth, H., Baumert, T., McKeating, J. A. & Chisari, F. V. (2007). Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. *J Virol* 81, 374-383.

Kaplan, D. E., Sugimoto, K., Newton, K., Valiga, M. E., Ikeda, F., Aytaman, A., Nunes, F. A., Lucey, M. R., Vance, B. A., Vonderheide, R. H., Reddy, K. R., McKeating, J. A. & Chang, K. M. (2007). Discordant role of CD4 T-cell response relative to neutralizing antibody and CD8 T-cell responses in acute hepatitis C. *Gastroenterology* 132, 654-666.

Kawaguchi, T., Yoshida, T., Harada, M., Hisamoto, T., Nagao, Y., Ide, T., Taniguchi, E., Kumemura, H., Hanada, S., Maeyama, M., Baba, S., Koga, H., Kumashiro, R., Ueno, T., Ogata, H., Yoshimura, A. & Sata, M. (2004). Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 165, 1499-1508.

Kawai, T. & Akira, S. (2008). Toll-like receptor and RIG-I-like receptor signaling. *Ann N Y Acad Sci* 1143, 1-20.

Keck, Z. Y., Li, S. H., Xia, J., von Hahn, T., Balfe, P., McKeating, J. A., Witteveldt, J., Patel, A. H., Alter, H., Rice, C. M. & Foung, S. K. (2009). Mutations in hepatitis C virus E2 located outside the CD81 binding sites lead to escape from broadly neutralizing antibodies but compromise virus infectivity. *J Virol* 83, 6149-6160.

Keir, M. E., Francisco, L. M. & Sharpe, A. H. (2007). PD-1 and its ligands in T-cell immunity. *Curr Opin Immunol* 19, 309-314.

Khakoo, S. I., Thio, C. L., Martin, M. P., Brooks, C. R., Gao, X., Astemborski, J., Cheng, J., Goedert, J. J., Vlahov, D., Hilgartner, M., Cox, S., Little, A. M., Alexander, G. J.,

Cramp, M. E., O'Brien, S. J., Rosenberg, W. M., Thomas, D. L. & Carrington, M. (2004). HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 305, 872-874.

Kim, K. I., Giannakopoulos, N. V., Virgin, H. W. & Zhang, D. E. (2004). Interferon-inducible ubiquitin E2, Ubc8, is a conjugating enzyme for protein ISGylation. *Mol Cell Biol* 24, 9592-9600.

Knapp, S., Warshow, U., Hegazy, D., Brackenbury, L., Guha, I. N., Fowell, A., Little, A. M., Alexander, G. J., Rosenberg, W. M., Cramp, M. E. & Khakoo, S. I. (2010). Consistent beneficial effects of killer cell immunoglobulin-like receptor 2DL3 and group 1 human leukocyte antigen-C following exposure to hepatitis C virus. *Hepatology* 51, 1168-1175.

Koutsoudakis, G., Kaul, A., Steinmann, E., Kallis, S., Lohmann, V., Pietschmann, T. & Bartenschlager, R. (2006). Characterization of the early steps of hepatitis C virus infection by using luciferase reporter viruses. *J Virol* 80, 5308-5320.

Koyama, A. H., Irie, H., Fukumori, T., Hata, S., Iida, S., Akari, H. & Adachi, A. (1998). Role of virus-induced apoptosis in a host defense mechanism against virus infection. *J Med Invest* 45, 37-45.

Kusano, F., Tanaka, Y., Marumo, F. & Sato, C. (2000). Expression of C-C chemokines is associated with portal and periportal inflammation in the liver of patients with chronic hepatitis C. *Lab Invest* 80, 415-422.

Lang, T., Lo, C., Skinner, N., Locarnini, S., Visvanathan, K. & Mansell, A. (2011) The Hepatitis B e antigen (HBeAg) targets and suppresses activation of the Toll-like receptor signaling pathway. *J Hepatol*. In press.

Lanzavecchia, A. & Sallusto, F. (2001). Regulation of T cell immunity by dendritic cells. *Cell* 106, 263-266.

Larrea, E., Riezu-Boj, J. I., Gil-Guerrero, L., Casares, N., Aldabe, R., Sarobe, P., Civeira, M. P., Heeney, J. L., Rollier, C., Verstrepen, B., Wakita, T., Borras-Cuesta, F., Lasarte, J. J. & Prieto, J. (2007). Upregulation of indoleamine 2,3-dioxygenase in hepatitis C virus infection. *J Virol* 81, 3662-3666.

Larrubia, J. R. (2011). Occult hepatitis B virus infection: a complex entity with relevant clinical implications. *World J Gastroenterol* 17, 1529-1530.

Larrubia, J. R., Benito-Martinez, S., Calvino, M., Sanz-de-Villalobos, E. & Parra-Cid, T. (2008). Role of chemokines and their receptors in viral persistence and liver damage during chronic hepatitis C virus infection. *World J Gastroenterol* 14, 7149-7159.

Larrubia, J. R., Benito-Martinez, S., Miquel-Plaza, J., Sanz-de-Villalobos, E., Gonzalez-Mateos, F. & Parra, T. (2009a). Cytokines - their pathogenic and therapeutic role in chronic viral hepatitis. *Rev Esp Enferm Dig* 101, 343-351.

Larrubia, J. R., Benito-Martinez, S., Miquel, J., Calvino, M., Sanz-de-Villalobos, E., Gonzalez-Praetorius, A., Albertos, S., Garcia-Garzon, S., Lokhande, M. & Parra-Cid, T. (2011). Bim-mediated apoptosis and PD-1/PD-L1 pathway impair reactivity of PD1(+)/CD127(-) HCV-specific CD8(+) cells targeting the virus in chronic hepatitis C virus infection. *Cell Immunol* 269, 104-114.

Larrubia, J. R., Benito-Martinez, S., Miquel, J., Calvino, M., Sanz-de-Villalobos, E. & Parra-Cid, T. (2009b). Costimulatory molecule programmed death-1 in the cytotoxic response during chronic hepatitis C. *World J Gastroenterol.* 15, 5129-5140.

Larrubia, J. R., Calvino, M., Benito, S., Sanz-de-Villalobos, E., Perna, C., Perez-Hornedo, J., Gonzalez-Mateos, F., Garcia-Garzon, S., Bienvenido, A. & Parra, T. (2007). The role of CCR5/CXCR3 expressing CD8+ cells in liver damage and viral control during persistent hepatitis C virus infection. *J Hepatol* 47, 632-641.

Lavanchy, D. (2009). The global burden of hepatitis C. *Liver Int* 29 Suppl 1, 74-81.

Lavillette, D., Bartosch, B., Nourrisson, D., Verney, G., Cosset, F. L., Penin, F. & Pecheur, E. I. (2006). Hepatitis C virus glycoproteins mediate low pH-dependent membrane fusion with liposomes. *J Biol Chem* 281, 3909-3917.

Lavillette, D., Morice, Y., Germanidis, G., Donot, P., Soulier, A., Pagkalos, E., Sakellariou, G., Intrator, L., Bartosch, B., Pawlotsky, J. M. & Cosset, F. L. (2005). Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. *J Virol* 79, 6023-6034.

Lavillette, D., Pecheur, E. I., Donot, P., Fresquet, J., Molle, J., Corbau, R., Dreux, M., Penin, F. & Cosset, F. L. (2007). Characterization of fusion determinants points to the involvement of three discrete regions of both E1 and E2 glycoproteins in the membrane fusion process of hepatitis C virus. *J Virol* 81, 8752-8765.

Lechner, F., Gruener, N. H., Urbani, S., Uggeri, J., Santantonio, T., Kammer, A. R., Cerny, A., Phillips, R., Ferrari, C., Pape, G. R. & Klenerman, P. (2000a). CD8+ T lymphocyte responses are induced during acute hepatitis C virus infection but are not sustained. *Eur J Immunol* 30, 2479-2487.

Lechner, F., Wong, D. K., Dunbar, P. R., Chapman, R., Chung, R. T., Dohrenwend, P., Robbins, G., Phillips, R., Klenerman, P. & Walker, B. D. (2000b). Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 191, 1499-1512.

Levrero, M., Pollicino, T., Petersen, J., Belloni, L., Raimondo, G. & Dandri, M. (2009). Control of cccDNA function in hepatitis B virus infection. *J Hepatol* 51, 581-592.

Li, K., Foy, E., Ferreon, J. C., Nakamura, M., Ferreon, A. C., Ikeda, M., Ray, S. C., Gale, M., Jr. & Lemon, S. M. (2005a). Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc Natl Acad Sci U S A* 102, 2992-2997.

Li, X. D., Sun, L., Seth, R. B., Pineda, G. & Chen, Z. J. (2005b). Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci U S A* 102, 17717-17722.

Liaw, Y. F., Brunetto, M. R. & Hadziyannis, S. (2010). The natural history of chronic HBV infection and geographical differences. *Antivir Ther* 15 Suppl 3, 25-33.

Lin, W., Kim, S. S., Yeung, E., Kamegaya, Y., Blackard, J. T., Kim, K. A., Holtzman, M. J. & Chung, R. T. (2006). Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. *J Virol* 80, 9226-9235.

Lindenbach, B. D., Evans, M. J., Syder, A. J., Wolk, B., Tellinghuisen, T. L., Liu, C. C., Maruyama, T., Hynes, R. O., Burton, D. R., McKeating, J. A. & Rice, C. M. (2005). Complete replication of hepatitis C virus in cell culture. *Science* 309, 623-626.

Liu, B., Liao, J., Rao, X., Kushner, S. A., Chung, C. D., Chang, D. D. & Shuai, K. (1998). Inhibition of Stat1-mediated gene activation by PIAS1. *Proc Natl Acad Sci U S A* 95, 10626-10631.

Liu, L. Q., Ilaria, R., Jr., Kingsley, P. D., Iwama, A., van Etten, R. A., Palis, J. & Zhang, D. E. (1999). A novel ubiquitin-specific protease, UBP43, cloned from leukemia fusion protein AML1-ETO-expressing mice, functions in hematopoietic cell differentiation. *Mol Cell Biol* 19, 3029-3038.

Liu, S., Yang, W., Shen, L., Turner, J. R., Coyne, C. B. & Wang, T. (2009). Tight junction proteins claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection. *J Virol* 83, 2011-2014.

Lodoen, M. B. & Lanier, L. L. (2006). Natural killer cells as an initial defense against pathogens. *Curr Opin Immunol* 18, 391-398.

Logvinoff, C., Major, M. E., Oldach, D., Heyward, S., Talal, A., Balfe, P., Feinstone, S. M., Alter, H., Rice, C. M. & McKeating, J. A. (2004). Neutralizing antibody response during acute and chronic hepatitis C virus infection. *Proc Natl Acad Sci U S A* 101, 10149-10154.

Lohmann, V., Korner, F., Koch, J., Herian, U., Theilmann, L. & Bartenschlager, R. (1999). Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 285, 110-113.

Longman, R. S., Talal, A. H., Jacobson, I. M., Albert, M. L. & Rice, C. M. (2004). Presence of functional dendritic cells in patients chronically infected with hepatitis C virus. *Blood* 103, 1026-1029.

Longman, R. S., Talal, A. H., Jacobson, I. M., Rice, C. M. & Albert, M. L. (2005). Normal functional capacity in circulating myeloid and plasmacytoid dendritic cells in patients with chronic hepatitis C. *J Infect Dis* 192, 497-503.

Lopes, A. R., Kellam, P., Das, A., Dunn, C., Kwan, A., Turner, J., Peppa, D., Gilson, R. J., Gehring, A., Bertoletti, A. & Maini, M. K. (2008). Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. *J Clin Invest* 118, 1835-1845.

Maini, M. K., Boni, C., Lee, C. K., Larrubia, J. R., Reignat, S., Ogg, G. S., King, A. S., Herberg, J., Gilson, R., Alisa, A., Williams, R., Vergani, D., Naoumov, N. V., Ferrari, C. & Bertoletti, A. (2000a). The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 191, 1269-1280.

Maini, M. K., Boni, C., Ogg, G. S., King, A. S., Reignat, S., Lee, C. K., Larrubia, J. R., Webster, G. J., McMichael, A. J., Ferrari, C., Williams, R., Vergani, D. & Bertoletti, A. (1999). Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. *Gastroenterology* 117, 1386-1396.

Maini, M. K., Reignat, S., Boni, C., Ogg, G. S., King, A. S., Malacarne, F., Webster, G. J. & Bertoletti, A. (2000b). T cell receptor usage of virus-specific CD8 cells and recognition of viral mutations during acute and persistent hepatitis B virus infection. *Eur J Immunol* 30, 3067-3078.

Maini, M. K. & Schurich, A. (2010). The molecular basis of the failed immune response in chronic HBV: therapeutic implications. *J Hepatol* 52, 616-619.

Manigold, T., Shin, E. C., Mizukoshi, E., Mihalik, K., Murthy, K. K., Rice, C. M., Piccirillo, C. A. & Rehermann, B. (2006). Foxp3+CD4+CD25+ T cells control virus-specific memory T cells in chimpanzees that recovered from hepatitis C. *Blood* 107, 4424-4432.

Manns, M. P., McHutchison, J. G., Gordon, S. C., Rustgi, V. K., Shiffman, M., Reindollar, R., Goodman, Z. D., Koury, K., Ling, M. & Albrecht, J. K. (2001). Peginterferon alfa-2b

plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 358, 958-965.

Manns, M. P. & Rambusch, E. G. (1999). Autoimmunity and extrahepatic manifestations in hepatitis C virus infection. *J Hepatol* 31 Suppl 1, 39-42.

Marie, I., Durbin, J. E. & Levy, D. E. (1998). Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor-7. *EMBO J* 17, 6660-6669.

Maruyama, T., Schodel, F., Iino, S., Koike, K., Yasuda, K., Peterson, D. & Milich, D. R. (1994). Distinguishing between acute and symptomatic chronic hepatitis B virus infection. *Gastroenterology* 106, 1006-1015.

McClary, H., Koch, R., Chisari, F. V. & Guidotti, L. G. (2000). Relative sensitivity of hepatitis B virus and other hepatotropic viruses to the antiviral effects of cytokines. *J Virol* 74, 2255-2264.

Meertens, L., Bertaux, C. & Dragic, T. (2006). Hepatitis C virus entry requires a critical postinternalization step and delivery to early endosomes via clathrin-coated vesicles. *J Virol* 80, 11571-11578.

Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R. & Tschopp, J. (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 437, 1167-1172.

Milich, D. & Liang, T. J. (2003). Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* 38, 1075-1086.

Milich, D. R. & Chisari, F. V. (1982). Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). I. H-2 restriction of the murine humoral immune response to the a and d determinants of HBsAg. *J Immunol* 129, 320-325.

Milich, D. R., Jones, J. E., Hughes, J. L., Price, J., Raney, A. K. & McLachlan, A. (1990). Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero?. *Proc Natl Acad Sci U S A* 87, 6599-6603.

Missale, G., Bertoni, R., Lamonaca, V., Valli, A., Massari, M., Mori, C., Rumi, M. G., Houghton, M., Fiaccadori, F. & Ferrari, C. (1996). Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. *J Clin Invest* 98, 706-714.

Molina, S., Castet, V., Fournier-Wirth, C., Pichard-Garcia, L., Avner, R., Harats, D., Roitelman, J., Barbaras, R., Graber, P., Ghersa, P., Smolarsky, M., Funaro, A., Malavasi, F., Larrey, D., Coste, J., Fabre, J. M., Sa-Cunha, A. & Maurel, P. (2007). The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. *J Hepatol* 46, 411-419.

Monazahian, M., Bohme, I., Bonk, S., Koch, A., Scholz, C., Grethe, S. & Thomssen, R. (1999). Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. *J Med Virol* 57, 223-229.

Montes-Cano, M. A., Caro-Oleas, J. L., Romero-Gomez, M., Diago, M., Andrade, R., Carmona, I., Aguilar Reina, J., Nunez-Roldan, A. & Gonzalez-Escribano, M. F. (2005). HLA-C and KIR genes in hepatitis C virus infection. *Hum Immunol* 66, 1106-1109.

Moradpour, D., Brass, V., Bieck, E., Friebe, P., Gosert, R., Blum, H. E., Bartenschlager, R., Penin, F. & Lohmann, V. (2004). Membrane association of the RNA-dependent

RNA polymerase is essential for hepatitis C virus RNA replication. *J Virol* 78, 13278-13284.

Moretta, L., Bottino, C., Pende, D., Vitale, M., Mingari, M. C. & Moretta, A. (2005). Human natural killer cells: Molecular mechanisms controlling NK cell activation and tumor cell lysis. *Immunol Lett* 100, 7-13.

Nakamoto, N., Cho, H., Shaked, A., Olthoff, K., Valiga, M. E., Kaminski, M., Gostick, E., Price, D. A., Freeman, G. J., Wherry, E. J. & Chang, K. M. (2009). Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. *PLoS Pathog* 5, e1000313.

Nakamoto, N., Kaplan, D. E., Coleclough, J., Li, Y., Valiga, M. E., Kaminski, M., Shaked, A., Olthoff, K., Gostick, E., Price, D. A., Freeman, G. J., Wherry, E. J. & Chang, K. M. (2008). Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. *Gastroenterology* 134, 1927-1937, 1937 e1921-1922.

Nascimbeni, M., Mizukoshi, E., Bosmann, M., Major, M. E., Mihalik, K., Rice, C. M., Feinstone, S. M. & Rehermann, B. (2003). Kinetics of CD4+ and CD8+ memory T-cell responses during hepatitis C virus rechallenge of previously recovered chimpanzees. *J Virol* 77, 4781-4793.

Neumann-Haefelin, C., McKiernan, S., Ward, S., Viazov, S., Spangenberg, H. C., Killinger, T., Baumert, T. F., Nazarova, N., Sheridan, I., Pybus, O., von Weizsacker, F., Roggendorf, M., Kelleher, D., Klenerman, P., Blum, H. E. & Thimme, R. (2006). Dominant influence of an HLA-B27 restricted CD8+ T cell response in mediating HCV clearance and evolution. *Hepatology* 43, 563-572.

Nowak, M. A. & Bangham, C. R. (1996). Population dynamics of immune responses to persistent viruses. *Science* 272, 74-79.

Oliviero, B., Varchetta, S., Paudice, E., Michelone, G., Zaramella, M., Mavilio, D., De Filippi, F., Bruno, S. & Mondelli, M. U. (2009). Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. *Gastroenterology* 137, 1151-1160, 1160 e1151-1157.

Paladino, N., Flores, A. C., Marcos, C. Y., Fainboim, H., Theiler, G., Arruvito, L., Williams, F., Middleton, D. & Fainboim, L. (2007). Increased frequencies of activating natural killer receptors are associated with liver injury in individuals who do not eliminate hepatitis C virus. *Tissue Antigens* 69 Suppl 1, 109-111.

Pavio, N. & Lai, M. M. (2003). The hepatitis C virus persistence: how to evade the immune system? *J Biosci* 28, 287-304.

Pawlotsky, J. M. (2004). Pathophysiology of hepatitis C virus infection and related liver disease. *Trends Microbiol* 12, 96-102.

Penna, A., Artini, M., Cavalli, A., Levrero, M., Bertoletti, A., Pilli, M., Chisari, F. V., Rehermann, B., Del Prete, G., Fiaccadori, F. & Ferrari, C. (1996). Long-lasting memory T cell responses following self-limited acute hepatitis B. *J Clin Invest* 98, 1185-1194.

Penna, A., Pilli, M., Zerbini, A., Orlandini, A., Mezzadri, S., Sacchelli, L., Missale, G. & Ferrari, C. (2007). Dysfunction and functional restoration of HCV-specific CD8 responses in chronic hepatitis C virus infection. *Hepatology* 45, 588-601.

Perrillo, R., Hou, J., Papatheodoridis, G. & Manns, M. (2010). Patient management and clinical decision making in HBV--aims of therapy and what we can achieve. *Antivir Ther* 15 Suppl 3, 45-51.

Pestka, J. M., Zeisel, M. B., Blaser, E., Schurmann, P., Bartosch, B., Cosset, F. L., Patel, A. H., Meisel, H., Baumert, J., Viazov, S., Rispeter, K., Blum, H. E., Roggendorf, M. & Baumert, T. F. (2007). Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci U S A* 104, 6025-6030.

Piazzolla, G., Tortorella, C., Schiraldi, O. & Antonaci, S. (2000). Relationship between interferon-gamma, interleukin-10, and interleukin-12 production in chronic hepatitis C and in vitro effects of interferon-alpha. *J Clin Immunol* 20, 54-61.

Pileri, P., Uematsu, Y., Campagnoli, S., Galli, G., Falugi, F., Petracca, R., Weiner, A. J., Houghton, M., Rosa, D., Grandi, G. & Abrignani, S. (1998). Binding of hepatitis C virus to CD81. *Science* 282, 938-941.

Pilli, M., Zerbini, A., Penna, A., Orlandini, A., Lukasiewicz, E., Pawlotsky, J. M., Zeuzem, S., Schalm, S. W., von Wagner, M., Germanidis, G., Lurie, Y., Esteban, J. I., Haagmans, B. L., Hezode, C., Lagging, M., Negro, F., Homburger, Y., Neumann, A. U., Ferrari, C. & Missale, G. (2007). HCV-specific T-cell response in relation to viral kinetics and treatment outcome (DITTO-HCV project). *Gastroenterology* 133, 1132-1143.

Ploss, A., Evans, M. J., Gaysinskaya, V. A., Panis, M., You, H., de Jong, Y. P. & Rice, C. M. (2009). Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 457, 882-886.

Polyak, S. J., Khabar, K. S., Paschal, D. M., Ezelle, H. J., Duverlie, G., Barber, G. N., Levy, D. E., Mukaida, N. & Gretch, D. R. (2001). Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. *J Virol* 75, 6095-6106.

Poordad, F., McCone, J., Jr., Bacon, B. R., Bruno, S., Manns, M. P., Sulkowski, M. S., Jacobson, I. M., Reddy, K. R., Goodman, Z. D., Boparai, N., DiNubile, M. J., Sniukiene, V., Brass, C. A., Albrecht, J. K. & Bronowicki, J. P. (2011). Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 364, 1195-1206.

Racanelli, V., Frassanito, M. A., Leone, P., Galiano, M., De Re, V., Silvestris, F. & Dammacco, F. (2006). Antibody production and in vitro behavior of CD27-defined B-cell subsets: persistent hepatitis C virus infection changes the rules. *J Virol* 80, 3923-3934.

Racanelli, V. & Rehermann, B. (2006). The liver as an immunological organ. *Hepatology* 43, S54-62.

Radziewicz, H., Ibegbu, C. C., Fernandez, M. L., Workowski, K. A., Obideen, K., Wehbi, M., Hanson, H. L., Steinberg, J. P., Masopust, D., Wherry, E. J., Altman, J. D., Rouse, B. T., Freeman, G. J., Ahmed, R. & Grakoui, A. (2007). Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression. *J Virol* 81, 2545-2553.

Rahman, F., Heller, T., Sobao, Y., Mizukoshi, E., Nascimbeni, M., Alter, H., Herrine, S., Hoofnagle, J., Liang, T. J. & Rehermann, B. (2004). Effects of antiviral therapy on the cellular immune response in acute hepatitis C. *Hepatology* 40, 87-97.

Rauch, A., Laird, R., McKinnon, E., Telenti, A., Furrer, H., Weber, R., Smillie, D. & Gaudieri, S. (2007). Influence of inhibitory killer immunoglobulin-like receptors and their

HLA-C ligands on resolving hepatitis C virus infection. *Tissue Antigens* 69 Suppl 1, 237-240.

Ray, S. C., Fanning, L., Wang, X. H., Netski, D. M., Kenny-Walsh, E. & Thomas, D. L. (2005). Divergent and convergent evolution after a common-source outbreak of hepatitis C virus. *J Exp Med* 201, 1753-1759.

Rehermann, B. (2009). Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 119, 1745-1754.

Rehermann, B., Ferrari, C., Pasquinelli, C. & Chisari, F. V. (1996). The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 2, 1104-1108.

Riordan, S. M., Skinner, N., Kurtovic, J., Locarnini, S. & Visvanathan, K. (2006). Reduced expression of toll-like receptor 2 on peripheral monocytes in patients with chronic hepatitis B. *Clin Vaccine Immunol* 13, 972-974.

Rong, L., Dahari, H., Ribeiro, R. M. & Perelson, A. S. (2010). Rapid emergence of protease inhibitor resistance in hepatitis C virus. *Sci Transl Med* 2, 30ra32.

Rushbrook, S. M., Ward, S. M., Unitt, E., Vowler, S. L., Lucas, M., Klenerman, P. & Alexander, G. J. (2005). Regulatory T cells suppress in vitro proliferation of virus-specific CD8+ T cells during persistent hepatitis C virus infection. *J Virol* 79, 7852-7859.

Rutebemberwa, A., Ray, S. C., Astemborski, J., Levine, J., Liu, L., Dowd, K. A., Clute, S., Wang, C., Korman, A., Sette, A., Sidney, J., Pardoll, D. M. & Cox, A. L. (2008). High-programmed death-1 levels on hepatitis C virus-specific T cells during acute infection are associated with viral persistence and require preservation of cognate antigen during chronic infection. *J Immunol* 181, 8215-8225.

Saito, T., Owen, D. M., Jiang, F., Marcotrigiano, J. & Gale, M., Jr. (2008). Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature* 454, 523-527.

Sakamoto, H., Kinjyo, I. & Yoshimura, A. (2000). The janus kinase inhibitor, Jab/SOCS-1, is an interferon-gamma inducible gene and determines the sensitivity to interferons. *Leuk Lymphoma* 38, 49-58.

Samuel, C. E. (2001). Antiviral actions of interferons. *Clin Microbiol Rev* 14, 778-809, table of contents.

Sarobe, P., Lasarte, J. J., Zabaleta, A., Arribillaga, L., Arina, A., Melero, I., Borras-Cuesta, F. & Prieto, J. (2003). Hepatitis C virus structural proteins impair dendritic cell maturation and inhibit in vivo induction of cellular immune responses. *J Virol* 77, 10862-10871.

Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawara, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N. & Taniguchi, T. (2000). Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. *Immunity* 13, 539-548.

Saunier, B., Triyatni, M., Ulianich, L., Maruvada, P., Yen, P. & Kohn, L. D. (2003). Role of the asialoglycoprotein receptor in binding and entry of hepatitis C virus structural proteins in cultured human hepatocytes. *J Virol* 77, 546-559.

Scarselli, E., Ansuini, H., Cerino, R., Roccasecca, R. M., Acali, S., Filocamo, G., Traboni, C., Nicosia, A., Cortese, R. & Vitelli, A. (2002). The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 21, 5017-5025.

Seifert, U., Liermann, H., Racanelli, V., Halenius, A., Wiese, M., Wedemeyer, H., Ruppert, T., Rispeter, K., Henklein, P., Sijts, A., Hengel, H., Kloetzel, P. M. & Rehermann, B. (2004). Hepatitis C virus mutation affects proteasomal epitope processing. *J Clin Invest* 114, 250-259.

Semmo, N., Lucas, M., Krashias, G., Lauer, G., Chapel, H. & Klenerman, P. (2006). Maintenance of HCV-specific T-cell responses in antibody-deficient patients a decade after early therapy. *Blood* 107, 4570-4571.

Sharpe, A. H., Wherry, E. J., Ahmed, R. & Freeman, G. J. (2007). The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol* 8, 239-245.

Shevach, E. M. (2009). Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity* 30, 636-645.

Shields, P. L., Morland, C. M., Salmon, M., Qin, S., Hubscher, S. G. & Adams, D. H. (1999). Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. *J Immunol* 163, 6236-6243.

Shin, E. C., Seifert, U., Kato, T., Rice, C. M., Feinstone, S. M., Kloetzel, P. M. & Rehermann, B. (2006). Virus-induced type I IFN stimulates generation of immunoproteasomes at the site of infection. *J Clin Invest* 116, 3006-3014.

Sillanpaa, M., Kaukinen, P., Melen, K. & Julkunen, I. (2008). Hepatitis C virus proteins interfere with the activation of chemokine gene promoters and downregulate chemokine gene expression. *J Gen Virol* 89, 432-443.

Smyk-Pearson, S., Tester, I. A., Klarquist, J., Palmer, B. E., Pawlotsky, J. M., Golden-Mason, L. & Rosen, H. R. (2008). Spontaneous recovery in acute human hepatitis C virus infection: functional T-cell thresholds and relative importance of CD4 help. *J Virol* 82, 1827-1837.

Sprengers, D., van der Molen, R. G., Binda, R., Kusters, J. G., de Man, R. A., Niesters, H. G., Schalm, S. W. & Janssen, H. L. (2007). In vivo immunization in combination with peg-interferon for chronic hepatitis B virus infection. *J Viral Hepat* 14, 743-749.

Sprengers, D., van der Molen, R. G., Kusters, J. G., Kwekkeboom, J., van der Laan, L. J., Niesters, H. G., Kuipers, E. J., De Man, R. A., Schalm, S. W. & Janssen, H. L. (2005). Flow cytometry of fine-needle-aspiration biopsies: a new method to monitor the intrahepatic immunological environment in chronic viral hepatitis. *J Viral Hepat* 12, 507-512.

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301-314.

Stegmann, K. A., Bjorkstrom, N. K., Veber, H., Ciesek, S., Riese, P., Wiegand, J., Hadem, J., Suneetha, P. V., Jaroszewicz, J., Wang, C., Schlaphoff, V., Fytili, P., Cornberg, M., Manns, M. P., Geffers, R., Pietschmann, T., Guzman, C. A., Ljunggren, H. G. & Wedemeyer, H. (2010). Interferon-alpha-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. *Gastroenterology* 138, 1885-1897.

Steinman, R. M., Hawiger, D. & Nussenzweig, M. C. (2003). Tolerogenic dendritic cells. *Annu Rev Immunol* 21, 685-711.

Stoop, J. N., van der Molen, R. G., Kuipers, E. J., Kusters, J. G. & Janssen, H. L. (2007). Inhibition of viral replication reduces regulatory T cells and enhances the antiviral immune response in chronic hepatitis B. *Virology* 361, 141-148.

Sugimoto, K., Ikeda, F., Stadanlick, J., Nunes, F. A., Alter, H. J. & Chang, K. M. (2003). Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 38, 1437-1448.

Szabo, G. & Dolganiuc, A. (2005). Subversion of plasmacytoid and myeloid dendritic cell functions in chronic HCV infection. *Immunobiology* 210, 237-247.

Tan, A. T., Koh, S., Goh, W., Zhe, H. Y., Gehring, A. J., Lim, S. G. & Bertoletti, A. (2010). A longitudinal analysis of innate and adaptive immune profile during hepatic flares in chronic hepatitis B. *J Hepatol* 52, 330-339.

Taylor, D. R., Shi, S. T., Romano, P. R., Barber, G. N. & Lai, M. M. (1999). Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 285, 107-110.

Tester, I., Smyk-Pearson, S., Wang, P., Wertheimer, A., Yao, E., Lewinsohn, D. M., Tavis, J. E. & Rosen, H. R. (2005). Immune evasion versus recovery after acute hepatitis C virus infection from a shared source. *J Exp Med* 201, 1725-1731.

Thimme, R., Bukh, J., Spangenberg, H. C., Wieland, S., Pemberton, J., Steiger, C., Govindarajan, S., Purcell, R. H. & Chisari, F. V. (2002). Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci U S A* 99, 15661-15668.

Thimme, R., Lohmann, V. & Weber, F. (2006). A target on the move: innate and adaptive immune escape strategies of hepatitis C virus. *Antiviral Res* 69, 129-141.

Thimme, R., Oldach, D., Chang, K. M., Steiger, C., Ray, S. C. & Chisari, F. V. (2001). Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 194, 1395-1406.

Timm, J., Lauer, G. M., Kavanagh, D. G., Sheridan, I., Kim, A. Y., Lucas, M., Pillay, T., Ouchi, K., Reyor, L. L., Schulze zur Wiesch, J., Gandhi, R. T., Chung, R. T., Bhardwaj, N., Klenerman, P., Walker, B. D. & Allen, T. M. (2004). CD8 epitope escape and reversion in acute HCV infection. *J Exp Med* 200, 1593-1604.

Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., Boulassel, M. R., Delwart, E., Sepulveda, H., Balderas, R. S., Routy, J. P., Haddad, E. K. & Sekaly, R. P. (2006). Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med* 12, 1198-1202.

Tscherne, D. M., Jones, C. T., Evans, M. J., Lindenbach, B. D., McKeating, J. A. & Rice, C. M. (2006). Time- and temperature-dependent activation of hepatitis C virus for low-pH-triggered entry. *J Virol* 80, 1734-1741.

Tsukuma, H., Hiyama, T., Tanaka, S., Nakao, M., Yabuuchi, T., Kitamura, T., Nakanishi, K., Fujimoto, I., Inoue, A., Yamazaki, H. & et al. (1993). Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 328, 1797-1801.

Urban, S., Schulze, A., Dandri, M. & Petersen, J. (2010). The replication cycle of hepatitis B virus. *J Hepatol* 52, 282-284.

Urbani, S., Amadei, B., Fisicaro, P., Tola, D., Orlandini, A., Sacchelli, L., Mori, C., Missale, G. & Ferrari, C. (2006a). Outcome of acute hepatitis C is related to virus-specific CD4 function and maturation of antiviral memory CD8 responses. *Hepatology* 44, 126-139.

Urbani, S., Amadei, B., Tola, D., Massari, M., Schivazappa, S., Missale, G. & Ferrari, C. (2006b). PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. *J Virol* 80, 11398-11403.

Veerapu, N. S., Raghuraman, S., Liang, T. J., Heller, T. & Rehermann, B. (2011). Sporadic reappearance of minute amounts of hepatitis C virus RNA after successful therapy stimulates cellular immune responses. *Gastroenterology* 140, 676-685 e671.

Visvanathan, K., Skinner, N. A., Thompson, A. J., Riordan, S. M., Sozzi, V., Edwards, R., Rodgers, S., Kurtovic, J., Chang, J., Lewin, S., Desmond, P. & Locarnini, S. (2007). Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 45, 102-110.

von Hahn, T., Yoon, J. C., Alter, H., Rice, C. M., Rehermann, B., Balfe, P. & McKeating, J. A. (2007). Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. *Gastroenterology* 132, 667-678.

Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., Murthy, K., Habermann, A., Krausslich, H. G., Mizokami, M., Bartenschlager, R. & Liang, T. J. (2005). Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 11, 791-796.

Wang, J., Zhao, J. H., Wang, P. P. & Xiang, G. J. (2008). Expression of CXC chemokine IP-10 in patients with chronic hepatitis B. *Hepatobiliary Pancreat Dis Int* 7, 45-50.

Webster, G. J., Reignat, S., Brown, D., Ogg, G. S., Jones, L., Seneviratne, S. L., Williams, R., Dusheiko, G. & Bertoletti, A. (2004). Longitudinal analysis of CD8+ T cells specific for structural and nonstructural hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. *J Virol* 78, 5707-5719.

Webster, G. J., Reignat, S., Maini, M. K., Whalley, S. A., Ogg, G. S., King, A., Brown, D., Amlot, P. L., Williams, R., Vergani, D., Dusheiko, G. M. & Bertoletti, A. (2000). Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 32, 1117-1124.

Wieland, S., Thimme, R., Purcell, R. H. & Chisari, F. V. (2004). Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* 101, 6669-6674.

Wieland, S. F. & Chisari, F. V. (2005). Stealth and cunning: hepatitis B and hepatitis C viruses. *J Virol* 79, 9369-9380.

Wieland, S. F., Guidotti, L. G. & Chisari, F. V. (2000). Intrahepatic induction of alpha/beta interferon eliminates viral RNA-containing capsids in hepatitis B virus transgenic mice. *J Virol* 74, 4165-4173.

Wu, J., Meng, Z., Jiang, M., Pei, R., Trippler, M., Broering, R., Bucchi, A., Sowa, J. P., Dittmer, U., Yang, D., Roggendorf, M., Gerken, G., Lu, M. & Schlaak, J. F. (2009). Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. *Hepatology* 49, 1132-1140.

Wu, J. F., Wu, T. C., Chen, C. H., Ni, Y. H., Chen, H. L., Hsu, H. Y. & Chang, M. H. (2010). Serum levels of interleukin-10 and interleukin-12 predict early, spontaneous hepatitis B virus e antigen seroconversion. *Gastroenterology* 138, 165-172 e161-163.

Wunschmann, S., Medh, J. D., Klinzmann, D., Schmidt, W. N. & Stapleton, J. T. (2000). Characterization of hepatitis C virus (HCV) and HCV E2 interactions with CD81 and the low-density lipoprotein receptor. *J Virol* 74, 10055-10062.

Xie, Q., Shen, H. C., Jia, N. N., Wang, H., Lin, L. Y., An, B. Y., Gui, H. L., Guo, S. M., Cai, W., Yu, H., Guo, Q. & Bao, S. (2009). Patients with chronic hepatitis B infection display

deficiency of plasmacytoid dendritic cells with reduced expression of TLR9. *Microbes Infect* 11, 515-523.

Xu, L. G., Wang, Y. Y., Han, K. J., Li, L. Y., Zhai, Z. & Shu, H. B. (2005). VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell* 19, 727-740.

Yerly, D., Heckerman, D., Allen, T. M., Chisholm, J. V., 3rd, Faircloth, K., Linde, C. H., Frahm, N., Timm, J., Pichler, W. J., Cerny, A. & Brander, C. (2008). Increased cytotoxic T-lymphocyte epitope variant cross-recognition and functional avidity are associated with hepatitis C virus clearance. *J Virol* 82, 3147-3153.

Zeisel, M. B., Cosset, F. L. & Baumert, T. F. (2008). Host neutralizing responses and pathogenesis of hepatitis C virus infection. *Hepatology* 48, 299-307.

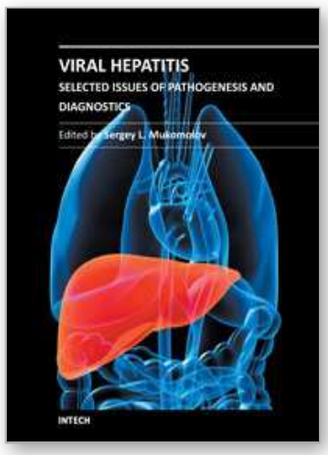
Zerbini, A., Pilli, M., Boni, C., Fisicaro, P., Penna, A., Di Vincenzo, P., Giuberti, T., Orlandini, A., Raffa, G., Pollicino, T., Raimondo, G., Ferrari, C. & Missale, G. (2008). The characteristics of the cell-mediated immune response identify different profiles of occult hepatitis B virus infection. *Gastroenterology* 134, 1470-1481.

Zhang, J. Y., Song, C. H., Shi, F., Zhang, Z., Fu, J. L. & Wang, F. S. (2010). Decreased ratio of Treg cells to Th17 cells correlates with HBV DNA suppression in chronic hepatitis B patients undergoing entecavir treatment. *PLoS One* 5, e13869.

Zhang, P., Zhong, L., Struble, E. B., Watanabe, H., Kachko, A., Mihalik, K., Virata-Theimer, M. L., Alter, H. J., Feinstone, S. & Major, M. (2009). Depletion of interfering antibodies in chronic hepatitis C patients and vaccinated chimpanzees reveals broad cross-genotype neutralizing activity. *Proc Natl Acad Sci U S A* 106, 7537-7541.

Zhao, C., Beaudenon, S. L., Kelley, M. L., Waddell, M. B., Yuan, W., Schulman, B. A., Huibregtse, J. M. & Krug, R. M. (2004). The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. *Proc Natl Acad Sci U S A* 101, 7578-7582.

Zhong, J., Gastaminza, P., Cheng, G., Kapadia, S., Kato, T., Burton, D. R., Wieland, S. F., Uprichard, S. L., Wakita, T. & Chisari, F. V. (2005). Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci U S A* 102, 9294-9299.



Viral Hepatitis - Selected Issues of Pathogenesis and Diagnostics
Edited by Dr. Sergey Mukomolov

ISBN 978-953-307-760-4
Hard cover, 152 pages
Publisher InTech
Published online 07, November, 2011
Published in print edition November, 2011

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.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Megha U. Lokhande, Joaquín Miquel, Selma Benito and Juan-R Larrubia (2011). HBV & HCV Immunopathogenesis, Viral Hepatitis - Selected Issues of Pathogenesis and Diagnostics, Dr. Sergey Mukomolov (Ed.), ISBN: 978-953-307-760-4, InTech, Available from: http://www.intechopen.com/books/viral-hepatitis-selected-issues-of-pathogenesis-and-diagnostics/hbv-hcv-immunopathogenesis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen