Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic Hepatitis B virus infection

Fabien Zoulim

Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic Hepatitis B virus infection

Fabien Zoulim

INSERM Unit 271

Insitut Universitaire de France

151 Cours Albert Thomas

69003, Lyon

France

Phone : 33-4 72 68 19 70

Fax : 33-4 72 68 19 71

E.mail : zoulim@lyon.inserm.fr

Abstract

Chronic hepatitis B virus (HBV) infections remain a major problem public health problem worldwide, as well as a therapeutic challenge for clinicians. This review focuses on the main viral and host determinants involved in HBV persistence in infected cells. The mechanism of HBV resistance to nucleoside analogs are described as well as the concept for multiple drug therapy and combination with immunostimulatory approaches.

Introduction

Despite the availability of an efficient vaccine, chronic HBV infection remains a major public health problem worlwide. Indeed, according to the World Health Organization, more than 400 million people are chronic carriers of the virus, and more than one billion have been in contact with HBV (Lee, 1997). Chronic carriers are exposed to the complications of the disease which include the development of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Ganem and Prince, 2004). The latter, usually develops after several decades of infection, but represents a major clinical problem in geographical areas where HBV is transmitted vertically. To combat or prevent these complications, several strategies are available. With respect to prophylaxis, HBV vaccine is safe and efficient. In endemic areas, it was shown that mass vaccination targeting not only adults but also infants was able to decrease the prevalence of HBV infection and the incidence of hepatocellular carcinoma (Chang et al., 1997). In patients who are already chronic carriers, the goals of antiviral therapy are to control viral replication and decrease liver damage to prevent the development of liver cirrhosis and subsequent hepatocellular carcinoma. In clinical practice, treatment relies mainly on the use of IFN alpha, or nucleoside analogs (Hoofnagle and Di Bisceglie, 1997), such as lamivuidne or adefovir dipivoxil. However, results of meta-analysis of IFN clinical trials showed that only a minority of patients are long term responders (approx 20%) (Wong et al., 1993). Its antiviral effect is also limited by the numerous side effects of this treatment. On the other hand, nucleoside analogs are well tolerated and exhibit an early and potent antiviral effect which is limited by the selection of resistant mutants during long-term therapy (Villeneuve et al., 2003; Zoulim, 2002). Therefore, antiviral therapy of chronic hepatitis B remains a clinical challenge.

The disease and the study models

As HBV replication does not lead by itself to a cytopathic effect, chronic hepatitis B is the result of the ongoing liver injury mediated by the T cell immune response targeting hepatocytes supporting a persistent HBV replication (Bertoletti and Naoumov, 2003; Ganem and Prince, 2004). Our knowledge of the pathobiology of HBV infection has come from clinical

studies and from experimental investigations performed in animal models. HBV belongs to the hepadnaviridae family that include animal viruses sharing the same replication strategy, ie woolly monkey hepatitis virus (WMHV) (Lanford et al., 1998), woodchuck hepatitis virus (WHV) (Summers et al., 1978), ground squirel hepatitis virus (GSHV) (Marion et al., 1980), artic squirel hepatitis virus (ASHV) (Testut et al., 1996), duck hepatitis B virus (DHBV) (Mason et al., 1980), heron hepatitis virus (HHV) (Sprengel et al., 1988), snow goose hepatitis virus (SGHV) (Sprengel et al., 1988), and crane hepatitis virus (Prassolov et al., 2003). Due to a narrow host range of the virus, the human HBV can only infect chimpanzee and several higher primates (Guidotti et al., 1999a; Lanford et al., 2000) as well as *tupaia belengeri*, a small primate which however does not support chronic infection (Walter et al., 1996). Other models have been developed to study human HBV biology. Transgenic mice harboring a given HBV transgene or a complete HBV genome have been very useful to study the in vivo effect of HBV proteins and their role in the development of hepatocellular carcinoma, ie the large envelope protein and the X protein (Chisari, 1995). Furthermore, this model allowed to study in detail the immunopathology of the disease and the influence of non cytolytic TH1 response in the clearance of HBV infection. However, a complete replication cycle does not occur in this model as mouse hepatocytes are not susceptible to HBV infection. Furthermore, cells harboring the HBV transgene and supporting HBV replication are not competent for cccDNA formation. Thus, only post-transcriptional events can be studied in this model. More recently, a model of humanized SCID mice that are deficient for the uPa gene and transplanted by human hepatocytes to allow animal survival has been developed. Upon repopulation of the mouse liver by transplanted hepatocytes, HBV infection can be initiated. (Petersen et al., 1998) However, due to the complex nature of this model, only specific questions can be addressed. .

Beside these models, other viruses belonging to the same family have been used to study the virological and host determinants involved in viral persistence during the natural history of the disease as well as during antiviral therapy. The two most used animal models have been the woodchuck infected with WHV and the Pekin duck infected with DHBV. They allowed to study the mechanism of viral clearance and viral persistence during antiviral

therapy. Moreover, the woodchuck represents a unique model for the study of the mechanism of virus induced carcinogenesis through the integration of viral genome sequences in the host genome and particularly in the vicinity of the c-Myc and N-myc oncogenes (Fourel et al., 1990; Moroy et al., 1986). Recent studies showed that molecular changes observed in the tumors of chronically WHV infected woodchucks suggest a progression of genetic alterations, including N-myc rearrangement, providing either a significant proliferative stimulation and/or a growth advantage in hepatocarcinogenesis of woodchucks with chronic WHV infection. These genetic alterations may be involved in the promotion of these tumors, ie late stage growth and vascularization of the tumors (Jacob et al., 2004).

These models have also contributed to the development of in vitro systems for the study of HBV genome replication. For a long time, it has been impossible to infect cell lines with HBV. In vitro studies relied on the analysis of HBV replication after transfection of hepatoma cell lines with appropriate vectors harboring replication competent HBV genomes. These systems allowed to perform genetic and functional studies to determine the role of HBV genes and proteins in the viral life cycle as well as to study the phenotype of HBV mutants selected in vivo (Fallows and Goff, 1995; Seeger et al., 1986; Seeger and Maragos, 1990; Seigneres et al., 2002). Permanent cell lines expressing continuously wild type HBV or drug resistant HBV genomes were generated to screen new antivirals (Fu and Cheng, 2000; Ladner et al., 1998; Ladner et al., 1997; Sells et al., 1987; Sureau et al., 1987). However, these systems allowed to study mainly post-transcriptional events, as the early steps of viral infection, ie viral entry and cccDNA formation, could not be analyzed. More recently, a cell line derived from human hepatocellular carcinoma was found to be susceptible for HBV infection and to support a full replication cycle including cccDNA formation (Gripon et al., 2002). This model may represent a major breakthrough for the understanding of viral entry, cccDNA and the biology of X protein as well as some aspects of cell curing in a chronically infected cells. Other approaches have been used to bypass the narrow cell range of HBV (limiting steps of cell infection) by using recombinant baculovirus or adenovirus vectors to infect hepatoma cell lines or primary hepatocytes (Delaney and Isom, 1998; Ren and Nassal, 2001).

Beside these cellular systems, several assays have been used to express an enzymatically active viral polymerase and study the cellular and virological components required for reverse transcription (Lanford et al., 1995; Wang and Seeger, 1992). Furthermore, these acellular assays were used to determine the mechanism of action of nucleoside analogs on wild type and drug resistant polymerases (Seigneres et al., 2002; Zoulim et al., 1996).

Viral replication and Persistence

HBV mainly infects hepatocytes, but other cell types including biliary epithelial cells, pancreas, kidney, skin, spleen, bone marrow, peripheral blood mononuclear cells, have been shown to harbor viral genome sequences and to support HBV expression to some extents. Whether these cells represent an extrahepatic reservoir for infectious HBV particle production remains controversial. The HBV replication cycle has been reviewed in detail (for further references, see (Ganem and Prince, 2004; Seeger and Mason, 2000), and is presented in figure 1.

After its interaction with a cellular receptor on the hepatocyte membrane, the HBV particle fuses with the membrane, and releases its nucleocapsid into the cytoplasm. The viral envelope proteins are shed during this process and the nucleocapsid migrates to the nucleus of the host cell. Viral DNA enters the nucleus where it is transformed into a supercoiled covalently closed circular proviral DNA molecule (cccDNA) (Tuttleman et al., 1986). This process requires the cleaving of the covalently attached viral polymerase from the negative strand and of the oligoribonucleotide sequence from the positive strand, completion of the positive strand, ligation of both strand extremities to form a covalently closed circular molecule, and then supercoiling of the viral DNA in the host chromatin. The exact enzymatic processes involved have not been elucidated so far. It was shown that the initial formation of cccDNA from incoming virions cannot be prevented using potent nucleoside analogs inhibiting the viral polymerase and plus strand DNA synthesis (Delmas et al., 2002; Fourel et al., 1994; Kock et al., 2003; Le Guerhier et al., 2000). This observation suggest that in patients receiving long-

term antiviral therapy, residual virions in the blood stream may still infect new cells despite the presence of the drug.

Interestingly, viral genome integration is not required for the viral life cycle. Indeed, unlike the proviral DNA of retroviruses, the hepatitis B cccDNA is not incorporated into the host genome. Its long half-life within infected hepatocytes, is the source of new infective virions when antiviral therapy is stopped. Reactivation of viral replication from cccDNA is the principal source of recurrence of clinical hepatitis in patients who have stopped using antiviral drugs or have been immunocompromised (organ transplantation, HIV co-infection etc).

The transcription of the viral pregenomic RNA from cccDNA by host RNA polymerase is driven by the HBV core promoter, in the nucleus of the host cell. The pregenomic RNA is encapsidated together with the viral polymerase. Within the newly formed nucleocapsid, the negative DNA strand is synthesized by the viral reverse transcriptase using the pregenomic RNA as a template. The first step involves covalent attachment of dGTP to the Tyr_{65} residue in the terminal protein domain of the polymerase after the interaction of the viral polymerase with the □ encapsidation signal on the pregenomic mRNA which is also used as a template for the addition of the first nucleotides (Hu et al., 1997; Wang et al., 1994; Wang and Seeger, 1992; Zoulim and Seeger, 1994). Following strand transfer, synthesis of minus strand DNA proceeds, and the viral pregenomic RNA is progressively degraded by the RnaseH domain of the polymerase. The positive DNA strand is then synthesised by the viral polymerase using the negative strand as a template.

The maturation of the capsid involves the dephosphorylation of the core proteins increasing its binding affinity for the recently formed double stranded DNA. At this stage, the capsid is mature and can be assembled with the viral envelope proteins present in the endoplasmic reticulum into a complete virion which can be released from infected cells into the circulation (Barrasa et al., 2001; Yu and Summers, 1994). An excess of the large envelope protein with respect to the other envelope proteins leads to the accumulation of viral particles within the lumen of the endoplasmic reticulum as it prevents trafficking through the Golgi and

disrupt normal shedding of viral particles (Lenhoff and Summers, 1994a; Lenhoff and Summers, 1994b). This may lead to a cytopathic effect such as the ground glass hepatocytes and induce oxidative stress in infected cells. Alternatively, the mature capsid can be recycled to the nucleus where it can be transformed into further copies of cccDNA molecules. In stably infected hepatocytes, the nucleus may contain between thirty and fifty copies of cccDNA. This serves as the reservoir for future replication cycles of the virus. In addition, the viral DNA can be directly integrated into the host DNA mainly via double stranded linear DNA and illegitimate recombination (Yang and Summers, 1995). Viral DNA thus integrated into the mammalian genome does not serve as a template for the production of pregenomic viral RNA. However, the presence of foreign viral DNA in the host genome may trigger the subsequent development of hepatocellular carcinoma.

In addition to the fact that wild type HBV replication usually does not lead to cell lysis, and that viral cccDNA has a long half-life, HBV genome variability is another major factor involved in viral persistence (Günther et al., 1999). Indeed, the viral polymerase lacks a 3',5' exonuclease that permits the excision of incorrectly inserted nucleotides. It has been estimated that in an individual infected with hepatitis B, 10^{10} incorrect nucleotides are incorporated into viral particles every day, providing a potential reservoir of genetic variants. This viral genome diversity leads to the classification of viral strains in genotypes, but some variants may also be selected depending on the selective pressure (ie replication fitness, sensitivity to antiviral drugs, escape from immune response etc...). Therefore, the virus circulates as a complex mixture of variants called quasi-species that evolves over time depending on the selective pressure (Figure 2). The selection of HBV mutants is limited by the fact that HBV open reading frames and regulatory sequences are partially overlapping; mutants in a given gene confering a replicative advantage may induce a lethal effect on the overlapping gene. For instance, some HBV polymerase mutants confering resistance to lamivudine may induce a stop codon in the overlaping surface gene leading to premature termination of the envelope protein translation.

Eight viral genotypes have been identified, known as genotypes A to H. From an epidemiological point of view, these genotypes are found in different geographical populations. Treatment responses to interferon-alpha apparently vary according to the viral genotype; infections with strains of genotypes A and B responding better than those with genotypes C or D respectively (Kao et al., 2000; Zhang et al., 1996). In contrast, most reports showed no obvious differences in treatment responses to nucleoside analogs between viral genotypes (Nafa et al., 2000; Pichoud et al., 1999; Seigneres et al., 2000; Westland et al., 2003).

Mechanisms of spontaneous or antiviral induced viral clearance

The mechanisms of viral clearance have been studied in detail by clinical studies as well as by the use of experimental models. Viral clearance requires the coordinated action of several components of the immune response (Bertoletti and Naoumov, 2003): 1) a cytotoxic TH1 response by CD8 positive cells recognizing infected hepatocytes expressing viral antigens ; 2) a non cytolytic TH1 response whereby the CD8 positive cells produce TH1 cytokines such as IFN gamma, TNF alpha, Interleukin 12 that exhibit a direct antiviral effect in the infected liver ; 3) the innate immune response mediated by NK and NKT cells ; 4) the production of neutralizing antibodies by B cells to prevent the infection of new hepatocytes ; 5) cell lysis and turn-over to generate new uninfected cells that will repopulate the liver.

While there is a concensus on the role of these different effectors, the order of these events is still debated. In transgenic mice and acutely infected chimpanzees, several studies have shown a major role of non cytolytic processes in the immune mediated clearance of viral infection (Cavanaugh et al., 1997; Guidotti et al., 1999b; Thimme et al., 2003). By contrast, in the woodchuck and duck models of acute infection, evidence has been obtained that cell lysis and hepatocyte turn-over are required to clear infection (Guo et al., 2000; Kajino et al., 1994; Summers et al., 2003).

The role of antivirals in the setting of chronic infection is to decrease viral production to prevent a new round of infection, but also to restore the specific CD4 and CD8 mediated immune response following the decrease in viral load in the infected host (Boni et al., 1998;

Boni et al., 2001). An HBV load of 10^7 copies/mL appears to be a threshold below which circulating multispecific HBV specific CD8+ Tcells are consistently detected (Webster et al., 2004). It is also possible that prolonged antiviral treatment may induce cell curing by inhibiting the intracellular recycling of cccDNA. It was also shown that viral persistence during long-term antiviral therapy in the duck and woodchuck models is linked to a long half-life of viral cccDNA (Fourel et al., 1994; Mason et al., 1998; Moraleda et al., 1997). Interestingly, several investigators have looked at the potential role of IFN gamma in the clearance of viral infection in chronically infected woodchucks in whom viral load had been decreased by nucleoside analog administration. By contrast to the observations made in transgenic mice, IFN gamma delivered via adenovirus vectors had no added benefit in terms of viral clearance (Jacquard et al., 2004; Lu et al., 2002).

Untill recently, the exact kinetics of viral cccDNA clearance has not been studied in detail in chronically infected patients. Indeed, due to the small size of liver biopsy samples and the relatively poor sensitivity of the Southern blot analysis of viral DNA, detailed studies on large number of patients were not possible. However, the persistence of cccDNA in the liver of infected individuals was demonstrated in untreated as well as in patients who received IFN alpha (Miller and Robinson, 1984; Yokosuka et al., 1985). More recently, PCR methodologies allowed to gain new insight in the persistence of this form of viral DNA. Results suggested that viral cccDNA may persist over decades even after the serological clearance of viral infection and that resolution of infection may not occur via sterilizing immunity but by the continuous control of viral infection over time. With the use of a real time PCR assay it was possible to demonstrate that levels of intrahepatic cccDNA decline during the sequential phases of the natural history of the disease but persist even in patients who lost serological markers of infection, consistent with the previous observations (Werle-Lapostolle et al., 2004). These data suggest that the reservoir of cccDNA may be the source of renewed viral replication and clinical reactivation in case of loss of immunological control, ie immunosuppression, organ transplantation, HIV co-infection.

Antiviral therapy with nucleoside analogs

The primary mechanism of action of nucleoside analogs is to inhibit viral polymerase activity. The structure of the compounds and their molecular mechanism of action have been reviewed recently (De Clercq, 1999; De Clercq, 2001). Figure 3 shows their site of action during the viral genome synthesis process.

Lamivudine inhibits viral reverse transcriptase activity, ie elongation of viral minus strand DNA, as it is a competitive inhibitor of its natural substrate, dCTP. Furthermore, after its incorporation in nascent viral DNA strand, it was shown to inhibit the addition of the next nucleotide and act as a DNA chain terminator (Severini et al., 1995; Zoulim et al., 1996). Adefovir inhibits the priming of reverse transcription by preventing the incorporation of dATP in the viral primer, and inhibits viral minus strand DNA elongation (Seigneres et al., 2001; Xiong et al., 1998). Entecavir was also shown to inhibit both the priming and elongation of viral minus strand DNA (Seifer et al., 1998). Emtricitabine exhibits a comparable inhibitory effect as lamivudine on the viral reverse transcriptase (Condreay et al., 1996; Condreay et al., 1994; Seigneres et al., 2002). Clevudine was shown to mainly inhibits viral plus strand DNA synthesis, ie DNA dependent DNA polymerase activity (Seigneres et al., 2002). Elvucitabine is a potent inhibitor of the reverse transcriptase activity and a DNA chain terminator (Le Guerhier et al., 2000). Few information is currently available regarding the mechanism of LdT (Telbivudine) (Standring et al., 2001) and 2',3'-dideoxy-3'-fluoroguanosine activity on HBV replication (Schroder et al., 1998).

The potency of the antiviral effect may depend on pharmacokinetics properties including, intestinal absorption, distribution into the infected liver, cellular uptake, efficiency of intracellular phosphorylation, half-life of the triphosphate form, excision from newly synthesized viral DNA, as well as on the selectivity index of the drug that may prevent the use of optimal doses to reach a more potent antiviral effect (Bridges and Cheng, 1995; Colacino, 1996; Lee and Chu, 2001; Urban et al., 2001). In the clinical setting, host determinants such as compliance, severity of the liver disease and associated disorders may also influence antiviral efficacy.

New insight in the kinetics of viral clearance has been obtained from phase II/III trials. Several studies performed in the setting of clinical trials of lamivudine (Nowak et al., 1996), adefovir dipivoxil (Tsiang et al., 1999) and entecavir (Wolters et al., 2002), have shown that schematically, viral clearance follows a biphasic decay. The first drop of viremia corresponds to the direct inhibitory effect of the nucleoside analogs on viral DNA synthesis and viral production. This is followed by a slower decline of viral load corresponding to the progressive clearance of infected cells and intrahepatic cccDNA. Other studies suggested a more complex pattern with a third phase, which may be linked to the activation of the specific anti-HBV immune response (Lewin et al., 2001). Interestingly, it was shown by several groups, that the drop in viral load induced by the administration of lamivudine or adefovir dipivoxil may be followed by the sequential restoration of the CD4 and CD8 immune response against HBV epitopes and then HBe seroconversion (Boni et al., 1998; Boni et al., 2001). It is noteworthy that several studies showed a correlation between the magnitude of the initial drop of viremia and the subsequent appearance of HBe seroconversion induced by lamivudine or adefovir dipivoxil (Gauthier et al., 1999; Werle et al., 2004).

A major issue in the treatment of chronic hepatitis B is to better understand the kinetics of loss of intrahepatic cccDNA as this may reflect the slower slope of the second or third phase. In this setting, a recent study performed in patients enrolled in a phase III trials of adefovir dipivoxil showed that a 48 week administration allowed to decrease serum viral load by approximately 4 $\log_{10}$, total intrahepatic viral DNA by approximately 2 $\log_{10}$, and intrahepatic cccDNA by 0.8 $\log_{10}$ (Werle-Lapostolle et al., 2004). Mathematical modeling assuming that the decrease of cccDNA levels in the liver is linear after the first year of therapy suggest that 14 years of therapy would be required to clear completely cccDNA from the liver. However, this does not take into account the specific immune response that would be able to control low levels of HBV infection prior to complete clearance (Webster et al., 2004). Furthermore, it was shown that the decline of HBsAg titer in serum paralleled that of intrahepatic cccDNA but that the number of cells did not decline significantly. In addition, the decrease of cccDNA was not

associated with baseline markers of cell lysis and turnover (ALT levels, histology activity index). Altogether, these results suggest that the decrease of intrahepatic cccDNA occured primarily via a non cytolytic pathway, ie the inhibition of viral DNA synthesis and intracellular recycling of cccDNA (Werle-Lapostolle et al., 2004). Further studies are ongoing to determine whether intrahepatic cccDNA may be a clinically useful marker to monitor antiviral therapy as well as to see if quantitative monitoring of serum viral antigens may be a surrogate marker for cccDNA.

Mechanism of viral drug resistance

The selection of drug resistant mutants depends on several factors (Figure 2). Viral persistence is the result of both the long half life of hepatocytes because of a defective immune response against infected cells, and the persistence of viral cccDNA in infected cells. As the viral polymerase is subjected to a spontaneous error rate, viral mutants are generated and accumulate during the natural history of the disease. The more viable variants in the context of the environmental pressure dominate. When an antiviral pressure is applied, the mutants exhibiting the best replication capacity in the presence of the drug are selected. The kinetics of emergence of these drug resistant mutants is usually slow. Indeed, several studies performed in animal models have shown that the time required to observe the emergence of a mutant, ie the increase in viral load associated with the dominance of the mutant, is linked to the need of a free liver space available for the mutants to spread in the liver (Zhou et al., 1999). The mutant spread depends also on its level of intrinsic resistance and on its replicative fitness. This may explain at least in part the difference of drug resistance rate observed with lamivudine (approx 20% at one year and up to 66% at 4 years) (Lai et al., 2003) and with adefovir dipivoxil (approx 2% at two year, and 3.9% at 3 years) (Xiong et al, EASL,Berlin, 2004). This also explains why the detection of resistant mutants using highly sensitive genotypic assays may allow to diagnose drug resistance prior to the rise in viral load (Nafa et al., 2000).

The mechanism of resistance mainly relies on the selection of viral polymerase mutants (Figure 4). Lamivudine resistance has been extensively studied. It was clearly shown that mutations in the YMDD motif within the catalytic site in the C domain of the reverse transcriptase, ie M204V and M204I, are conferring a high level of resistance to lamivudine (>1000 fold) both in vitro, in enzymatic and cellular assays, and in vivo (Allen et al., 1998; Fu et al., 1999). Interestingly it was shown that these mutants have a decreased enzymatic activity and replication capacity. Later, associated mutations were described in the neighboring B domain, ie V173L and L180M, and were shown to be compensatory mutations that restore partially the replication capacity of the C domain mutants (Delaney et al., 2003; Fu and Cheng, 1998; Seigneres et al., 2002). Modeling of the interaction between lamivudine triphosphate and the viral polymerase based on predicted tridimensional structure and homologies with the HIV reverse transcriptase indicated that the C domain mutations may prevent the correct interaction with lamivudine triphosphate, mainly by steric hindrance (Das et al., 2001; Lee and Chu, 2001).

Adefovir may select polymerase resistant mutants in another viral polymerase domain, ie the D domain. The N236T mutant confers a 5 to 10 fold resistance to adefovir in vitro. This weak resistance profile may explain the delayed emergence of this mutant. Furthermore, modeling of the interaction with the viral polymerase suggests that the flexibility of the chemical structure of adefovir and its close homology with its natural substrate may also explain the slow emergence of the mutant. It was also suggested that this mutant may interfere with the nucleotide binding pocket of the reverse transcriptase (Bartholomeusz et al., 2004). Another mutant, the A181V located in the B domain of the polymerase has been described to confer a similar level of resistance in vitro, but its clinical significance remains unclear.

Based on available data, resistance to entecavir seems to follow a more complex pattern. Preliminary results suggest that entecavir resistance occurs in patients who previously failed lamivudine treatment and that the resistance mutations occur on viral genomes already harbouring the lamivudine resistance mutations (Colonno et al, EASL, Berlin, 2004). Further

longitudinal studies with a longer follow up are required to see whether drug resistance may also occur in patients who were not previously experienced with nucleoside analogs.

Emtricitabine was shown to select for the same drug resistant mutants as lamivudine, although at a lower rate in phase II and III trials (9% at one year, and 18% at two years) (Gish et al., 2002). Preliminary data obtained in phase II trials with Telbivudine show the same trend with the selection of a M204I mutation in less than 10% of the patients after one year of treatment. No data are available yet with clevudine, but experiments performed in the woodchuck models seem to suggest that resistance to clevudine follow the same pattern as lamivudine.

It is also important to note, that the definition of resistance used in these different reports varies from a study to another. In some studies, viral polymerase gene sequence was analyzed in all patients in whom HBV DNA was detectable by PCR, while in other studies the viral polymerase sequence was analyzed only in patients who showed a significant increase of viral load during therapy (at least one log10 by comparison with the nadir value).

With famciclovir, the prodrug of penciclovir, drug resistance followed two patterns. One was a primary non response which was not associated with pre-existing resistant mutants or less susceptible viral genotypes, but most likely to the poor phosphorylation levels in infected cells, in vivo. The second, was the selection of famciclovir resistant mutants, with mutations located in the B domain, ie L18OM, or the C domain, ie V206I (Bartholomeusz and Locarnini, 1997; Pichoud et al., 1999). The selection of the L180M mutant by famciclovir was also shown to predispose to a more rapid selection of lamivudine resistance when patients were switched from famciclovir to lamivudine after failure of the former drug (Mutimer et al., 2000; Seigneres et al., 2000).

The resistance phenomenon to clevudine (L-FMAU) has been mainly studied in the woodchuck model (Yamamoto et al., 2002). In this model, resistance of WHV to lamivudine, and possibly L-FMAU, is associated primarily with mutations in the B region. This contrasts with HBV, where resistance to lamivudine results primarily from mutations of the YMDD motif in the C region. These data also suggested that the WHV mutants resistant to lamivudine are

also resistant to clevudine, as also oberved with HBV (Seigneres et al., 2002), and vice versa. It appears that the pattern of WHV polymerase mutations is not a perfect match to mutations developing in HBV patients, although the woodchuck remains a useful model for characterizing the biology of emergence of drug-resistant variants.

Phenotypic analysis of HBV clinical isolates

In the case of HIV therapy, drug resistance testing is now recommended to guide the choice of new drug regimens after the first or multiple treatment failures. In addition to genotypic assays, several phenotypic assays have been developed for HIV and are currently used in clinical practice to monitor drug resistance.

Until recently, no phenotypic drug susceptibility assay had been developed for HBV because of the small number of drug used in clinic, that rendered such assays unnecessary yet and the technical challenge associated with the molecular biology of the virus. However, with the current and future development of new anti-HBV molecules, phenotypic drug susceptibility testing may become an important tool for the management of patients infected with resistant HBV isolates and for the evaluation of the effect of new antivirals on clinical isolates circulating in the population. Until recently, the analysis of the phenotype of naturally occurring or drug induced HBV mutants has relied either on PCR-mediated transfer of HBV genome cassettes or on site directed mutagenesis within a well established replication-competent laboratory strain (Zoulim, 2001). This allowed for instance to confirm that the M204V/I $\pm$ L180M mutants selected *in vivo* in patients during antiviral therapy were indeed conferring resistance to lamivudine (Allen et al., 1998). Moreover, Gunther *et al.* described an original and efficient method for PCR amplification of full-length HBV genomes that was meant to facilitate the analysis of naturally occurring HBV variants, but actually led to low levels of intracellular replication after transfection of hepatoma cell lines (Günther et al., 1995).

New strategies for the rapid cloning of HBV genomes isolated from patients into plasmidic vectors and the basis of the first phenotypic assays capable of assessing HBV drug susceptibility *in vitro* and evaluate new antivirals on circulating viral strains have been recently

described (Durantel et al., 2004; Yang et al., 2004). These assays allowed to characterize resistance to adefovir dipivoxil in liver transplanted patients, and to shown that this viral strain harboring the N236T mutation remained susceptible to lamivudine which was further demonstrated in vivo in the clinical situation (Villeneuve et al., 2003). One assay also allows the phenotypic analysis of multiple clones mimicking the viral quasi-species circulating in vivo in the patient (Durantel et al., 2004).

With the rapid development of new nucleoside analogs with anti-HBV activity and the anticipation of the selection of new drug resistant mutants and/or multiple drug resistant mutants, these phenotypic assays may prove to be clinically relevant either for treatment monitoring, or for screening of new antivirals targeting the resistant strains

---

Prevention of and combating drug resistance

The rationale for combination therapy relies on the following evidence that are based on experimental findings in the hepadnavirus models and on HIV therapy experience (Richman, 2000) : 1) simple mutants pre-exist as shown by longitudinal studies of viral polymerase gene sequence, 2) genetic variants harboring multiple mutations have less chance to pre-exist or to occur, 3) re-treatment leads to a rapid re-emergence of resistant mutants although wild type virus re-emerges after first treatment interruption ; the same applies to sequential treatment selecting for partially cross resistant strains, ie famciclovir and lamivudine, 4) cccDNA represents a genetic archive for a more rapid re-selection of resistance.

The optimal antiviral regimen may combine drugs with a different mechanism of action on viral replication, lacking cross-resistance and exhibiting antiviral synergy. This theoretical type of combinations should reduce the risk of selection of drug resistant mutants because of the inhibition of viral replication and the selective pressure exerted on the different viral strains that compose the quasi-species (Figure 5). For instance, it was shown that the combination of lamivudine and famciclovir hastens the kinetics of viral clearance although no long term follow up was available to determine its effect on the prevention of drug resistance (Lau et al., 2000).

With the development and evaluation of newer drugs acting at different steps of the viral genome replication, it will be easier to choose the best combination relying on drugs with different mechanism of action, for instance drugs with potent anti-priming activity, drugs inhibiting viral minus strand DNA and others inhibiting plus strand DNA synthesis. The combination of such compounds was found to be either additive and more rarely synergistic in polymerase assays (reverse transcriptase activity) (Seigneres et al., 2003) as well as in tissue culture experiments (viral DNA synthesis) or in chronically infected woodchucks (Colledge et al., 2000; Colledge et al., 1997; Korba, 1996; Korba et al., 2000; Seigneres et al., 2003). Although some of the combinations based on the inhibition of all three steps of viral genome replication inhibited more potently viral DNA synthesis than the single drugs, none were able to completely prevent the initial formation of viral cccDNA following de novo infection of hepatocytes, or to clear cccDNA once chronic infection of hepatocytes has been established in tissue culture (Seigneres et al., 2003). However, such combinations by inhibiting more potently viral DNA synthesis may delay the onset of viral resistance by limiting the chance of a given mutation to occur.

Furthermore, results of cross-resistance studies on the main lamivudine and adefovir resistant strains are now becoming available (Table 1). Interestingly, it was shown that lamivudine resistant mutants are not sensitive to L-pyrimidine analogs such as emtricitabine, clevudine, elvucitabine, telbivudine while they remain susceptible to purine analogs such as adefovir, tenofovir, and to some extent to entecavir (Delaney et al., 2001; Fu and Cheng, 2000; Levine et al., 2002; Menne et al., 2002; Seigneres et al., 2002). The adefovir resistant strains are also sensitive to lamivudine, emtricitabine, and entecavir, and to some extent to tenofovir (Angus et al., 2003; Villeneuve et al., 2003). This may be due to the fact the lamivudine and the adefovir resistance mutations are not located in the same viral polymerase domains.

This should allow the evaluation of rational combinations taking into account the mechanism of action of the drugs as well as their antiviral activity and cross resistance profile,

to better suppress viral replication and prevent the selection of resistant mutants within the viral quasi-species (Zoulim, 2003).

Another approach is to decrease viral load by a combination of nucleoside analogs and then to break immune tolerance using an immunomodulatory approach to enhance the restoration of the immune response associated with the decrease in viral load. Such strategy has not proven beneficial when a combination of lamivudine with standard IFN alpha or pegylated IFN alpha was assessed using different schedules. Several studies are ongoing to evaluate the combination of adefovir dipivoxil and pegylated IFN alpha. The use of specific hepadnavirus vaccine has been evaluated in addition to nucleoside analog administration. One study in the woodchuck model using clevudine followed by WHV surface protein vaccine (Menne et al., 2002), and another in the duck model using adefovir and a surface gene DNA vaccine showed a trend for an enhanced clearance of viral infection in the combination arms (Le Guerhier et al., 2003). However, other studies using different protocols did not show an added benefit of the immunomodulatory approach. Other approaches rely on the induction of a non specific TH1 response in the infected liver using viruses targeting the liver (Zhou et al., 2000) or on the delivery of TH1 cytokines (Jacquard, 2004), once suppression of viral DNA synthesis has been obtained with antivirals. However, these approaches did not show an enhanced antiviral effect. Thus, more knowledge on the molecular and cellular events associated with viral clearance is required to design better trials combining nucleoside analogs and immune modulators.

Perspective

The better knowledge of viral replication should allow the development of new therapeutic concepts (Figure 1). In this view, novel inhibitors of viral replication are being evaluated in experimental systems. These includes myristilated pre-S1 peptides to compete with the interaction of the virion envelope proteins and the hepatocyte receptor (Urban and Gripon, 2002), peptides or recombinant core proteins exhibiting a trans dominant negative effect on nucleocapsid assembly (von Weizsacker et al., 1999), non nucleosides inhibitors that inhibit the correct assembly and packaging of pregenomic RNA, iminosugars to inhibit the

correct glycosylation process of viral envelope proteins and the maturation of virions. For instance, phenylpropenamide derivatives were shown to inhibit HBV replication independently of an interference with the viral polymerase activity (Delaney et al., 2002; King et al., 1998). It was suggested that these compounds may inhibit the packaging of pregenomic RNA, which in turn may explain their antiviral activity on both wild type and lamivudine resistant strains. Other compounds belonging to a different family of heteroaryldihydropyrimidines were shown to be non nucleosidic inhibitors of HBV replication, by a mechanism inhibiting viral capsid formation and increased degradation of the core protein (Deres et al., 2003). It was also recently suggested that APOBEC3G, a cellular cytidine deaminase, might interfere with the packaging of pregenomic RNA (Turelli et al., 2004). Stability of viral nucleocapsid may also be affected by cytokines such as IFN alpha (Schultz and Chisari, 1999; Schultz et al., 1999), but also by phosphorylation of cdc2 kinase like recognition motif (Barrasa et al., 2001). Other approaches also gave interesting results with the use of small molecules, such as peptides to block the interaction of core protein with the large envelope protein (Böttcher et al., 1998). Another family of compounds, the iminosugars, that inhibit cellular alpha glucosidase, were shown to interfere with envelope protein glycosylation and folding, and therefore to inhibit viral particle secretion (Block and Jordan, 2001; Block et al., 1998).

All these approaches have the advantage of targeting steps that are not dependent on the reverse transcriptase and should therefore become relevant for the treatment of resistance to nucleoside analog and/or to prevent the development of resistance when administered in association with nucleoside analogs. Other approaches based on the use of antisense approaches to target regions of the HBV genome that are essential for replication, ie antisense oligonucleotides (Offensperger et al., 1993; Robaczewska et al., 2001), peptide nucleic acids (PNAs) (Robaczewska et al, submitted), and short interfering RNAs (siRNAs) (Klein et al., 2003; McManus and Sharp, 2002; Shlomai and Shaul, 2003), are also being evaluated in cell culture systems, although one drawback/limitation could be the delivery of such nucleic acids to infected cells in vivo (von Weizsäcker et al., 1997). Together with the newer immuno-modulatory interventions relying on vaccine therapy, these new experimental concepts will

need to be evaluated in the relevant animal models prior to clinical trials. This should pave the way of new anti-HBV treatments relying on combination of agents which are less likely to trigger viral resistance.

Figures

Figure 1. The HBV replication cycle and site of action of nucleoside analogs

The figure shows all the major steps required in the viral replication cycle. Nucleoside(tide) analogs mainly inhibit the viral minus strand DNA synthesis (reverse transcription), and plus strand DNA synthesis (DNA dependent DNA polymerase activity) within viral nucleocapsids. These drugs do not affect directly viral cccDNA which is maintained in the nucleus of infected cells, therefore requiring long-term treatment for a sustained control of viral replication.

Figure 2. Mechanism of selection of drug resistant mutants

The main factors involved in the selection of escape mutants are indicated : i) the long half-life of hepatocytes and viral cccDNA; ii) the HBV genome variability ; iii) the selective pressure.

Figure 3. Mechanism of action of the main nucleoside analogs that inhibit viral polymerase activity.

Nucleoside analogs may inhibit viral polymerase activity by interfering with the priming of reverse transcription, elongation of viral minus strand DNA, elongation of plus strand DNA.

PMEA : adefovir ; DAPD : amdoxovir ; 3TC : lamivudine ; FTC : emtricitabine ; LFd4C : elvucitabine ; L-FMAU : clevudine.

Figure 4. Map of the viral polymerase gene and position of the main drug resistant mutants.

The main genetic and functional domains of the viral polymerase are indicated. The main mutations confering resistance to nucleoside analogs are indicated.

Figure 5. The concept of combination therapy and antiviral pressure on HBV quasi-species.

HBV circulates as a viral quasi-species. In this scheme, wild type virus is predominant, while lamivudine and adefovir resistant strains are found as minor variants. Monotherapy with one or the other nucleoside analog may therefore lead to the selection of a drug resistant mutant. Combination of the two antiviral agents which do not show cross-resistance should prevent the selection of these variants. The only possibility is the selection of a multiple drug resistant strain which should be much less frequent as this would require the presence of a complex pattern of mutations on the same viral genome. The same concept applies to any other polymerase inhibitors which do not share the same resistance profile.

Table

Table 1. Summary of cross-resistance testing.

The table shows the nucleoside analogs that are active (+) or inactive (-) on wild type, lamivudine resistant, or adefovir resistant strains. Adapted from Zoulim, 2003.

References

Allen, M.I., Deslauriers, M., Andrews, C.W., Tipples, G.A., Walters, K.A., Tyrell, D.L.J., Brown, N. and Condreay, L.D. (1998) Identification and characterization of mutations in hepatitis B virus resistant to Lamivudine. Hepatology 27, 1670-1677.

Angus, P., Vaughan, R., Xiong, S., Yang, H., Delaney, W., Gibbs, C., Brosgart, C., Colledge, D., Edwards, R., Ayres, A., Bartholomeusz, A. and Locarnini, S. (2003) Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 125(2), 292-7.

Barrasa, M.I., Guo, J.T., Saputelli, J., Mason, W.S. and Seeger, C. (2001) Does a cdc2 kinase-like recognition motif on the core protein of hepadnaviruses regulate assembly and disintegration of capsids? J Virol 75(4), 2024-8.

Bartholomeusz, A. and Locarnini, S. (1997) Mutations in the hepatitis B virus polymerase gene that are associated with resistance to famciclovir and lamivudine. International Antiviral News 5, 123-124.

Bartholomeusz, A., Tehan, B.G. and Chalmers, D.K. (2004) Comparisons of the HBV and HIV polymerase, and antiviral resistance mutations. Antivir Ther 9(2), 149-60.

Bertoletti, A. and Naoumov, N.V. (2003) Translation of immunological knowledge into better treatments of chronic hepatitis B. J Hepatol 39(1), 115-24.

Block, T.M. and Jordan, R. (2001) Iminosugars as possible broad spectrum anti hepatitis virus agents: the glucovirs and alkovirs. Antivir Chem Chemother 12(6), 317-25.

Block, T.M., Lu, X., Mehta, A.S., Blumberg, B.S., Tennant, B., Ebling, M., Korba, B., Lansky, D.M., Jacob, G.S. and Dwek, R.A. (1998) Treatment of chronic hepadnavirus infection in a woodchuck animal model with an inhibitor of protein folding and trafficking. Nat Med 4(5), 610-4.

Boni, C., Bertoletti, A., Penna, A., Cavalli, A., Pilli, M., Urbani, S., Scognamiglio, P., Boehme, R., Panebianco, R., Fiaccadori, F. and Ferrari, C. (1998) Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B [see comments]. J Clin Invest 102(5), 968-75.

Boni, C., Penna, A., Ogg, G.S., Bertoletti, A., Pilli, M., Cavallo, C., Cavalli, A., Urbani, S., Boehme, R., Panebianco, R., Fiaccadori, F. and Ferrari, C. (2001) Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology 33(4), 963-71.

Böttcher, B., Tsuji, N., Takahashi, H., Dyson, M., Zhao, S., Crowther, R.A. and Murray, K. (1998) Peptides that block hepatitis B virus assembly: analysis by cryomicroscopy, mutagenesis and transfection. EMBO J 17, 6839-6845.

Bridges, E. and Cheng, Y. (1995) Use of novel b-L(-)-nucleoside analogues for treatment and prevention of chronic hepatitis B virus infection and hepatocellular carcinoma. Progress in liver diseases. Eds, Boyer, JL and Ockner, RK 13, 231-245.

Cavanaugh, V.J., Guidotti, L. and Chisari, F.V. (1997) Interleukine-12 inhibits hepatitis B virus replication in transgenic mice. J. Virol. 71, 3236-3243.

Chang, M.-H., Chen, C.-J., Lai, M.-S., Hsu, H.-M., Wu, T.-C., Kong, M.-S., Liang, D.-C., Shau, W.-Y. and Chen, D.-S. (1997) Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. N. Engl. J. Med. 336, 1855-1859.

Chisari, F. (1995) Hepatitis B virus transgenic mice: insights into the virus and the disease. Hepatology 22, 1316-1325.

Colacino, J. (1996) Mechanisms for the anti-hepatitis B virus activity and mitochondrial toxicity of fialuridine (FIAU). Antiviral Res. 29, 125-139.

Colledge, D., Civitico, G., Locarnini, S. and Shaw, T. (2000) In vitro antihepadnaviral activities of combinations of penciclovir, lamivudine, and adefovir [In Process Citation]. Antimicrob Agents Chemother 44(3), 551-60.

Colledge, D., Locarnini, S. and Shaw, T. (1997) Synergistic inhibition of hepadnaviral replication by lamivudine in combination with penciclovir in vitro. Hepatology 26, 216-225.

Condreay, L., Condreay, J., Jansen, R., Paff, M. and Averett, D. (1996) (-)-cis-5-fluoro-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)cytosine (524W91) inhibits hepatitis B virus replication in primary human hepatocytes. Antimicrob; Agents Chemother. 40, 520-523.

Condreay, L., Jansen, R., Powdrill, T., Johnson, L., Selleseth, D., Paff, M., Daluge, S., Painter, G., Furman, P., Ellis, M. and Averett, D. (1994) Evaluation of the potent anti-HBV agent (-)cis-5-fluoro-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl) cytosine in a novel in vivo model. Antimicrob. Agents Chemother. 38, 616-619.

Das, K., Xiong, X., Yang, H., Westland, C.E., Gibbs, C.S., Sarafianos, S.G. and Arnold, E. (2001) Molecular modeling and biochemical characterization reveal the mechanism of hepatitis B virus polymerase resistance to lamivudine (3TC) and emtricitabine (FTC). J Virol 75(10), 4771-9.

De Clercq, E. (1999) Perspectives for the treatment of hepatitis B virus infections. Int J Antimicrob Agents 12(2), 81-95.

De Clercq, E. (2001) Antiviral drugs: current state of the art. J Clin Virol 22(1), 73-89.

Delaney, W.E. and Isom, H.C. (1998) Hepatitis B virus replication in human HepG2 cells mediated by hepatitis B virus recombinant baculovirus. Hepatology 28, 1134-1146.

Delaney, W.E.t., Edwards, R., Colledge, D., Shaw, T., Furman, P., Painter, G. and Locarnini, S. (2002) Phenylpropenamide derivatives AT-61 and AT-130 inhibit replication of wild-type and lamivudine-resistant strains of hepatitis B virus in vitro. Antimicrob Agents Chemother 46(9), 3057-60.

Delaney, W.E.t., Edwards, R., Colledge, D., Shaw, T., Torresi, J., Miller, T.G., Isom, H.C., Bock, C.T., Manns, M.P., Trautwein, C. and Locarnini, S. (2001) Cross-resistance testing of antihepadnaviral compounds using novel recombinant baculoviruses which encode drug-resistant strains of hepatitis B virus. Antimicrob Agents Chemother 45(6), 1705-13.

Delaney, W.E.t., Yang, H., Westland, C.E., Das, K., Arnold, E., Gibbs, C.S., Miller, M.D. and Xiong, S. (2003) The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication in vitro. J Virol 77(21), 11833-41.

Delmas, J., Schorr, O., Jamard, C., Gibbs, C., Trepo, C., Hantz, O. and Zoulim, F. (2002) Inhibitory effect of adefovir on viral DNA synthesis and covalently closed circular DNA formation in duck hepatitis B virus-infected hepatocytes in vivo and in vitro. Antimicrob Agents Chemother 46(2), 425-33.

Deres, K., Schroder, C.H., Paessens, A., Goldmann, S., Hacker, H.J., Weber, O., Kramer, T., Niewohner, U., Pleiss, U., Stoltefuss, J., Graef, E., Koletzki, D., Masantschek, R.N., Reimann, A., Jaeger, R., Gross, R., Beckermann, B., Schlemmer, K.H., Haebich, D. and Rubsamen-Waigmann, H. (2003) Inhibition of hepatitis B virus replication by drug-induced depletion of nucleocapsids. Science 299(5608), 893-6.

Durantel, D., Durantel, S., Werle, B., Pichoud, C., Trépo, C. and Zoulim, F. (2004) Hepatology in press.

Fallows, D. and Goff, S. (1995) Mutations in the e sequences of human hepatitis B virus affect both RNA encapsidation and reverse transcription. J. Virol. 69, 3067-3073.

Fourel, G., Trepo, C., Bougueleret, L., Henglein, B., Ponzetto, A., Tiollais, P. and Buendia, M.A. (1990) Frequent activation of N-myc genes by hepadnavirus insertion in woodchuck liver tumors. Nature 347, 294-298.

Fourel, I., Saputelli, J., Schaffer, P. and Mason, W.S. (1994) The carbocyclic analog of 2'-deoxyguanosine induces a prolonged inhibition of duck hepatitis B virus DNA synthesis in primary hepatocyte cultures and in the liver. J. Virol. 68, 1059-1065.

Fu, L. and Cheng, Y.-C. (1998) Role of additional mutations outside the YMDD motif of hepatitis B virus polymerase in L(-)SddC (3TC) resistance. Biochem Pharmacol 55, 1567-1572.

Fu, L. and Cheng, Y.C. (2000) Characterization of novel human hepatoma cell lines with stable hepatitis B virus secretion for evaluating new compounds against lamivudine- and penciclovir-resistant virus [In Process Citation]. Antimicrob Agents Chemother 44(12), 3402-7.

Fu, L., Liu, S.H. and Cheng, Y.C. (1999) Sensitivity of L-(-)2,3-dideoxythiacytidine resistant hepatitis B virus to other antiviral nucleoside analogues. Biochem Pharmacol 57(12), 1351-9.

Ganem, D. and Prince, A.M. (2004) Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 350(11), 1118-29.

Gauthier, J., Bourne, E.J., Lutz, M.W., Crowther, L.M., Dienstag, J.L., Brown, N.A. and Condreay, L.D. (1999) Quantitation of Hepatitis B Viremia and Emergence of YMDD Variants in Patients with Chronic Hepatitis B Treated with Lamivudine. J Infect Dis 180(6), 1757-1762.

Gish, R.G., Leung, N.W., Wright, T.L., Trinh, H., Lang, W., Kessler, H.A., Fang, L., Wang, L.H., Delehanty, J., Rigney, A., Mondou, E., Snow, A. and Rousseau, F. (2002) Dose range study of pharmacokinetics, safety, and preliminary antiviral activity of emtricitabine in adults with hepatitis B virus infection. Antimicrob Agents Chemother 46(6), 1734-40.

Gripon, P., Rumin, S., Urban, S., Le Seyec, J., Glaise, D., Cannie, I., Guyomard, C., Lucas, J., Trepo, C. and Guguen-Guillouzo, C. (2002) Infection of a human hepatoma cell line by hepatitis B virus. Proc Natl Acad Sci U S A 99(24), 15655-60.

Guidotti, L.G., Borrow, P., Brown, A., McClary, H., Koch, R. and Chisari, F.V. (1999a) Noncytopathic clearance of lymphocytic choriomeningitis virus from the hepatocyte. J Exp Med 189(10), 1555-64.

Guidotti, L.G., Rochford, R., Chung, J., Shapiro, M., Purcell, R. and Chisari, F.V. (1999b) Viral clearance without destruction of infected cells during acute HBV infection. Science 284(5415), 825-9.

Günther, S., Fischer, L., Pult, I., Sterneck, M. and Will, H. (1999) Naturally occuring variants of hepatitis B virus. Adv Virus Res 52, 25-137.

Günther, S., Li, B.-C., Miska, S., Kruger, D., Meisel, H. and Will, H. (1995) A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. J. Virol. 69, 5437-5444.

Guo, J.T., Zhou, H., Liu, C., Aldrich, C., Saputelli, J., Whitaker, T., Barrasa, M.I., Mason, W.S. and Seeger, C. (2000) Apoptosis and regeneration of hepatocytes during recovery from transient hepadnavirus infections [In Process Citation]. J Virol 74(3), 1495-505.

Hoofnagle, J.H. and Di Bisceglie, A.M. (1997) The treatment of chronic viral hepatitis. N. Engl. J. Med. 336, 347-356.

Hu, J., Toft, D. and Seeger, C. (1997) Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. EMBO J. 16, 59-68.

Jacob, J.R., Sterczer, A., Toshkov, I.A., Yeager, A.E., Korba, B.E., Cote, P.J., Buendia, M.A., Gerin, J.L. and Tennant, B.C. (2004) Integration of woodchuck hepatitis and N-myc rearrangement determine size and histologic grade of hepatic tumors. Hepatology 39(4), 1008-16.

Jacquard, A.C., Nassal, M., Pichoud, C., Ren, S., Schultz, U., Guerret, S., Chevallier, M., Werle, B., Peyrol, S., Jamard, C., Rimsky, L.T., Trepo, C. and Zoulim, F. (2004) Effect of a combination of clevudine and emtricitabine with adenovirus-mediated delivery of gamma interferon in the woodchuck model of hepatitis B virus infection. Antimicrob Agents Chemother 48(7), 2683-92.

Jacquard, C. (2004) Antimicrob Agents Chemother in press.

Kajino, K., Jilbert, A., Saputelli, J., Aldrich, C., Cullen, J. and Mason, W. (1994) Woodchuck hepatitis virus infections: very rapid recovery after a prolonged viremia and infection of virtually every hepatocyte. J. Virol. 68, 5792-5803.

Kao, J.H., Wu, N.H., Chen, P.J., Lai, M.Y. and Chen, D.S. (2000) Hepatitis B genotypes and the response to interferon therapy. J Hepatol 33(6), 998-1002.

King, R.W., Ladner, S.K., Miller, T.J., Zaifert, K., Perni, R.B., Conway, S.C. and Otto, M.J. (1998) Inhibition of human hepatitis B virus replication by AT-61, a phenylpropenamide

derivative, alone and in combination with (-)b-L-2',3'-dideoxy-3'-thiacytidine. Antimicrob. Agents Chemother. 42, 3179-3186.

Klein, C., Bock, C.T., Wedemeyer, H., Wustefeld, T., Locarnini, S., Dienes, H.P., Kubicka, S., Manns, M.P. and Trautwein, C. (2003) Inhibition of hepatitis B virus replication in vivo by nucleoside analogues and siRNA. Gastroenterology 125(1), 9-18.

Kock, J., Baumert, T.F., Delaney, W.E.t., Blum, H.E. and von Weizsacker, F. (2003) Inhibitory effect of adefovir and lamivudine on the initiation of hepatitis B virus infection in primary tupaia hepatocytes. Hepatology 38(6), 1410-8.

Korba, B.E. (1996) In vitro evaluation of combination therapies against hepatitis B virus replication. Antiviral Res 29(1), 49-51.

Korba, B.E., Cote, P., Hornbuckle, W., Schinazi, R., Gerin, J.L. and Tennant, B.C. (2000) Enhanced antiviral benefit of combination therapy with lamivudine and famciclovir against WHV replication in chronic WHV carrier woodchucks. Antiviral Res 45(1), 19-32.

Ladner, S., Miller, T.J. and King, R.W. (1998) The M539V polymerase variant of human hepatitis B virus demonstrates resistance to 2'-deoxy-3'-thiacytidine and a reduced ability to synthesize viral DNA. Antimicrob. Agents Chemother. 42, 2128-2131.

Ladner, S.K., Otto, M.J., Barker, C.S., Zaifert, K., Wang, G.-H., Guo, J.-T., Seeger, C. and King, R.W. (1997) Inducible expression of human hepatitis B virus (HBV) instably transfected hepatoblastoma cells : a novel system for screening potential inhibitors of HBV replication. Antimicrob. Agents Chemother. 41, 1715-1720.

Lai, C.L., Dienstag, J., Schiff, E., Leung, N.W., Atkins, M., Hunt, C., Brown, N., Woessner, M., Boehme, R. and Condreay, L. (2003) Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 36(6), 687-96.

Lanford, R., Notvall, L. and Beames, B. (1995) Nucleotide priming and reverse transcriptase activity of hepatitis B virus polymerase expressed in insect cells. J. Virol. 69, 4431-4439.

Lanford, R.E., Chavez, D., Brasky, K.M., Burns, R.B. and Ricco-Hesse, R. (1998) Isolation of a hepadnavirus from the woolly monkey, a new world primate. Proc. Natl. Acad. Sci. USA 95, 5757-5761.

Lanford, R.E., Chavez, D., Rico-Hesse, R. and Mootnick, A. (2000) Hepadnavirus infection in captive gibbons. J Virol 74(6), 2955-9.

Lau, G.K., Tsiang, M., Hou, J., Yuen, S., Carman, W.F., Zhang, L., Gibbs, C.S. and Lam, S. (2000) Combination therapy with lamivudine and famciclovir for chronic hepatitis B-infected Chinese patients: a viral dynamics study [see comments]. Hepatology 32(2), 394-9.

Le Guerhier, F., Pichoud, C., Guerret, S., Chevallier, M., Jamard, C., Hantz, O., Li, X.Y., Chen, S.H., King, I., Trepo, C., Cheng, Y.C. and Zoulim, F. (2000) Characterization of the antiviral effect of 2',3'-dideoxy-2', 3'- didehydro-beta-L-5-fluorocytidine in the duck hepatitis B virus infection model. Antimicrob Agents Chemother 44(1), 111-22.

Le Guerhier, F., Thermet, A., Guerret, S., Chevallier, M., Jamard, C., Gibbs, C.S., Trepo, C., Cova, L. and Zoulim, F. (2003) Antiviral effect of adefovir in combination with a DNA vaccine in the duck hepatitis B virus infection model. J Hepatol 38(3), 328-34.

Lee, K. and Chu, C.K. (2001) Molecular modeling approach to understanding the mode of action of L-nucleosides as antiviral agents. Antimicrob Agents Chemother 45(1), 138-44.

Lee, W.M. (1997) Hepatitis B virus infection. N. Engl. J. Med. 337, 1733-1745.

Lenhoff, R. and Summers, J. (1994a) Construction of avian hepadnavirus variants with enhanced replication and cytopathicity in primary hepatocytes. J. Virol. 68, 5706-5713.

Lenhoff, R. and Summers, J. (1994b) Coordinate regulation of replication and virus assembly by the large envelope protein of an avian hepadnavirus. J. Virol. 68, 4565-4571.

Levine, S., Hernandez, D., Yamanaka, G., Zhang, S., Rose, R., Weinheimer, S. and Colonno, R.J. (2002) Efficacies of entecavir against lamivudine-resistant hepatitis B virus

replication and recombinant polymerases in vitro. Antimicrob Agents Chemother 46(8), 2525-32.

Lewin, S.R., Ribeiro, R.M., Walters, T., Lau, G.K., Bowden, S., Locarnini, S. and Perelson, A.S. (2001) Analysis of hepatitis B viral load decline under potent therapy: Complex decay profiles observed. Hepatology 34(5), 1012-20.

Lu, M., Lohrengel, B., Hilken, G., Kemper, T. and Roggendorf, M. (2002) Woodchuck gamma interferon upregulates major histocompatibility complex class I transcription but is unable to deplete woodchuck hepatitis virus replication intermediates and RNAs in persistently infected woodchuck primary hepatocytes. J Virol 76(1), 58-67.

Marion, P.L., Oshiro, L.S., Regnery, D.C., Scullard, G.H. and Robinson, W.S. (1980) A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. Proc. Natl. Acad. Sci. USA 77, 2941-2945.

Mason, W.S., Cullen, J., Moraleda, G., Saputelli, J., Aldrich, C.E., Miller, D.S., Tennant, B., Frick, L., Averett, D., Condreay, L.D. and Jilbert, A.R. (1998) Lamivudine therapy of WHV-infected woodchucks. Virology 245, 18-32.

Mason, W.S., Seal, G. and Summers, J. (1980) Virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. J. Virol. 36, 829-836.

McManus, M.T. and Sharp, P.A. (2002) Gene silencing in mammals by small interfering RNAs. Nat Rev Genet 3(10), 737-47.

Menne, S., Roneker, C.A., Korba, B.E., Gerin, J.L., Tennant, B.C. and Cote, P.J. (2002) Immunization with surface antigen vaccine alone and after treatment with 1-(2-fluoro-5-methyl-beta-L-arabinofuranosyl)-uracil (L-FMAU) breaks humoral and cell-mediated immune tolerance in chronic woodchuck hepatitis virus infection. J Virol 76(11), 5305-14.

Miller, R.H. and Robinson, W.S. (1984) Hepatitis B virus DNA in nuclear and cytoplasmic fractions of infected human liver. Virology 137, 390-399.

Moraleda, G., Saputelli, J., Aldrich, C.E., Averett, D., Condreay, L. and Mason, W.S. (1997) Lack of effect of antiviral therapy in nondividing hepatocyte cultures on the closed circular DNA of woodchuck hepatitis virus. J. Virol. 71, 9392-9399.

Moroy, T., Marchio, A., Etiemble, J., Trepo, C., Tiollais, P. and Buendia, M. (1986) Rearrangement and enhanced expression of c-myc in hepatocellular carcinoma of hepatitis virus infected woodchucks. Nature 324, 276-279.

Mutimer, D., Pillay, D., Cook, P., Ratcliffe, D., O'Donnell, K., Dowling, D., Shaw, J., Elias, E. and Cane, P.A. (2000) Selection of multiresistant hepatitis B virus during sequential nucleoside-analogue therapy. J Infect Dis 181(2), 713-6.

Nafa, S., Ahmed, S., Tavan, D., Pichoud, C., Berby, F., Stuyver, L., Johnson, M., Merle, P., Abidi, H., Trepo, C. and Zoulim, F. (2000) Early Detection of Viral Resistance by Determination of Hepatitis B Virus Polymerase Mutations in Patients Treated by Lamivudine for Chronic Hepatitis B. Hepatology 32(5), 1078-1088.

Nowak, M., Bonhoeffer, S., Hill, A., Boehme, R., Thomas, H. and McDade, H. (1996) Viral dynamics in hepatitis B virus infection. Proc. Natl. Acad. Sci. USA 93, 4398-4402.

Offensperger, W., Offensperger, S., Walter, E., Teubner, K., Igloi, G., Blum, H. and Gerok, W. (1993) In vivo inhibition of duck hepatitis B virus replication and gene expression by phosphorothioate modified antisense oligodeoxynucleotides. EMBO J. 12, 1257-1262.

Petersen, J., Dandri, M., Gupta, S. and Rogler, C.E. (1998) Liver repopulation with xenogenic hepatocytes in B and T cell-deficient mice leads to chronic hepadnavirus infection and clonal growth of hepatocellular carcinoma. Proc Natl Acad Sci U S A 95(1), 310-5.

Pichoud, C., Seignères, B., Wang, Z., Trepo, C. and Zoulim, F. (1999) Transient selection of a hepatitis B virus polymerase gene mutant associated with a decreased replication capacity and famciclovir resistance. Hepatology 29, 230-237.

Prassolov, A., Hohenberg, H., Kalinina, T., Schneider, C., Cova, L., Krone, O., Frolich, K., Will, H. and Sirma, H. (2003) New hepatitis B virus of cranes that has an unexpected broad host range. J Virol 77(3), 1964-76.

Ren, S. and Nassal, M. (2001) Hepatitis B virus (HBV) virion and covalently closed circular DNA formation in primary tupaia hepatocytes and human hepatoma cell lines upon

HBV genome transduction with replication-defective adenovirus vectors. J Virol 75(3), 1104-16.

Richman, D.D. (2000) The impact of drug resistance on the effectiveness of chemotherapy for chronic hepatitis B. Hepatology 32(4 Pt 1), 866-7.

Robaczewska, M., Guerret, S., Remy, J.S., Chemin, I., Offensperger, W.B., Chevallier, M., Behr, J.P., Podhajska, A.J., Blum, H.E., Trepo, C. and Cova, L. (2001) Inhibition of hepadnaviral replication by polyethylenimine-based intravenous delivery of antisense phosphodiester oligodeoxynucleotides to the liver. Gene Ther 8(11), 874-81.

Schroder, I., Holmgren, B., Oberg, M. and Lofgren, B. (1998) Inhibition of human and duck hepatitis B virus by 2',3'-dideoxy-3'-fluoroguanosine in vitro. Antiviral Res 37(1), 57-66.

Schultz, U. and Chisari, F.V. (1999) Recombinant duck interferon gamma inhibits duck hepatitis B virus replication in primary hepatocytes. J Virol 73(4), 3162-8.

Schultz, U., Summers, J., Staeheli, P. and Chisari, F.V. (1999) Elimination of duck hepatitis B virus RNA-containing capsids in duck interferon-alpha-treated hepatocytes [In Process Citation]. J Virol 73(7), 5459-65.

Seeger, C., Ganem, D. and Varmus, H.E. (1986) Biochemical and genetic evidence for the hepatitis B virus replication strategy. Science 232, 477-484.

Seeger, C. and Maragos, J. (1990) Identification and characterization of the woodchuck hepatitis virus origin of DNA replication. J.Virol. 64, 16-23.

Seeger, C. and Mason, W.S. (2000) Hepatitis B virus biology. Microbiol Mol Biol Rev 64(1), 51-68.

Seifer, M., Hamatake, R.K., Colonno, R.J. and Standring, D.N. (1998) In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobucavir. Antimicrob. Agents Chemother. 42, 3200-3208.

Seigneres, B., Aguesse-Germon, S., Pichoud, C., Vuillermoz, I., Jamard, C., Trepo, C. and Zoulim, F. (2001) Duck hepatitis B virus polymerase gene mutants associated with resistance to lamivudine have a decreased replication capacity in vitro and in vivo. J Hepatol 34(1), 114-22.

Seigneres, B., Martin, P., Werle, B., Schorr, O., Jamard, C., Rimsky, L., Trepo, C. and Zoulim, F. (2003) Effects of pyrimidine and purine analog combinations in the duck hepatitis B virus infection model. Antimicrob Agents Chemother 47(6), 1842-52.

Seigneres, B., Pichoud, C., Ahmed, S.S., Hantz, O., Trepo, C. and Zoulim, F. (2000) Evolution of Hepatitis B Virus Polymerase Gene Sequence during Famciclovir Therapy for Chronic Hepatitis B. J Infect Dis 181(4), 1221-1233.

Seigneres, B., Pichoud, C., Martin, P., Furman, P., Trepo, C. and Zoulim, F. (2002) Inhibitory activity of dioxolane purine analogs on wild-type and lamivudine-resistant mutants of hepadnaviruses. Hepatology 36(3), 710-22.

Sells, M.A., Chen, M. and Acs, G. (1987) Production of hepatitis B virus particles in hepG2 cells transfected with cloned hepatitis B virus DNA. Proc. Natl. Acad. Sci. USA 84, 1005-1009.

Severini, A., Liu, X.-Y., Wilson, J. and Tyrell, D. (1995) Mechanism of inhibition of duck hepatitis B virus polymerase by (-)-b-L-2',3'-dideoxy-3'-thyacytidine. Antimicrob. Agents Chemother. 39, 1430-1435.

Shlomai, A. and Shaul, Y. (2003) Inhibition of hepatitis B virus expression and replication by RNA interference. Hepatology 37(4), 764-70.

Sprengel, R., Kaleta, E.F. and Will, H. (1988) Isolation and characterization of a hepatitis B virus endemic in herons. J. Virol. 62, 3832-3839.

Standring, D.N., Bridges, E.G., Placidi, L., Faraj, A., Loi, A.G., Pierra, C., Dukhan, D., Gosselin, G., Imbach, J.L., Hernandez, B., Juodawlkis, A., Tennant, B., Korba, B., Cote, P., Cretton-Scott, E., Schinazi, R.F., Myers, M., Bryant, M.L. and Sommadossi, J.P. (2001) Antiviral beta-L-nucleosides specific for hepatitis B virus infection. Antivir Chem Chemother 12 Suppl 1, 119-29.

Summers, J., Jilbert, A.R., Yang, W., Aldrich, C.E., Saputelli, J., Litwin, S., Toll, E. and Mason, W.S. (2003) Hepatocyte turnover during resolution of a transient hepadnaviral infection. Proc Natl Acad Sci U S A 100(20), 11652-9.

Summers, J., Smolec, J.M. and Snyder, R. (1978) A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc. Natl. Acad. Sci. USA 75, 4533-4537.

Sureau, C., Romet-Lemonne, J., Mullins, J.I. and Essex, M. (1987) Production of hepatitis B virus by a differentiated human hepatoma cell line after transfection with cloned circular HBV DNA. Cell 47, 37-47.

Testut, P., Renard, C.A., Terradillos, O., Vitvitski-Trepo, L., Tekaia, F., Degott, C., Blake, J., Boyer, B. and Buendia, M.A. (1996) A new hepadnavirus endemic in arctic ground squirrels in Alaska. J Virol 70(7), 4210-9.

Thimme, R., Wieland, S., Steiger, C., Ghrayeb, J., Reimann, K.A., Purcell, R.H. and Chisari, F.V. (2003) CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 77(1), 68-76.

Tsiang, M., Rooney, J.F., Toole, J.J. and Gibbs, C.S. (1999) Biphasic clearance kinetics of hepatitis B virus from patients during adefovir dipivoxil therapy. Hepatology 29(6), 1863-9.

Turelli, P., Mangeat, B., Jost, S., Vianin, S. and Trono, D. (2004) Inhibition of hepatitis B virus replication by APOBEC3G. Science 303(5665), 1829.

Tuttleman, J., Pourcel, C. and Summers, J. (1986) Formation of the pool of covalently closed circular viral DNA in hepadnavirus infected cells. Cell 47, 451-460.

Urban, S., Fischer, K.P. and Tyrrell, D.L. (2001) Efficient pyrophosphorolysis by a hepatitis B virus polymerase may be a primer-unblocking mechanism. Proc Natl Acad Sci U S A 98(9), 4984-9.

Urban, S. and Gripon, P. (2002) Inhibition of duck hepatitis B virus infection by a myristoylated pre-S peptide of the large viral surface protein. J Virol 76(4), 1986-90.

Villeneuve, J.P., Durantel, D., Durantel, S., Westland, C., Xiong, S., Brosgart, C.L., Gibbs, C.S., Parvaz, P., Werle, B., Trepo, C. and Zoulim, F. (2003) Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. J Hepatol 39(6), 1085-9.

von Weizsacker, F., Kock, J., Wieland, S., Offensperger, W.B. and Blum, H.E. (1999) Dominant negative mutants of the duck hepatitis B virus core protein interfere with RNA pregenome packaging and viral DNA synthesis. Hepatology 30(1), 308-15.

von Weizsäcker, G., Wieland, S., Köck, J., Offensperger, W.-B., Offensperger, S., Moradpour, D. and Blum, H.E. (1997) Gene therapy for chronic viral hepatitis : ribozymes, antisense oligonucleotides, and dominant negative mutants. Hepatology 26, 251-255.

Walter, E., Keist, R., Niederöst, B., Pult, I. and Blum, H. (1996) Hepatitis B virus infection of tupaia hepatocytes in vitro and in vivo. Hepatology 24, 1-5.

Wang, G., Zoulim, F., Leber, E., Kitson, J. and Seeger, C. (1994) The role of RNA in enzymatic activity of the reverse transcriptase of hepatitis B viruses. J. Virol. 68, 8437-8442.

Wang, G.H. and Seeger, C. (1992) The reverse transcriptase of hepatitis B virus acts a protein primer for viral DNA synthesis. Cell 71, 633-670.

Webster, G.J., Reignat, S., Brown, D., Ogg, G.S., Jones, L., Seneviratne, S.L., Williams, R., Dusheiko, G. and 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(11), 5707-19.

Werle, B., Cinquin, K., Marcellin, P., Pol, S., Maynard, M., Trepo, C. and Zoulim, F. (2004) Evolution of hepatitis B viral load and viral genome sequence during adefovir dipivoxil therapy. J Viral Hepat 11(1), 74-83.

Werle-Lapostolle, B., Bowden, S., Locarnini, S., Wursthorn, K., Petersen, J., Lau, G., Trepo, C., Marcellin, P., Goodman, Z., Delaney, W.E.t., Xiong, S., Brosgart, C.L., Chen, S.S., Gibbs, C.S. and Zoulim, F. (2004) Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 126(7), 1750-8.

Westland, C., Delaney, W.t., Yang, H., Chen, S.S., Marcellin, P., Hadziyannis, S., Gish, R., Fry, J., Brosgart, C., Gibbs, C., Miller, M. and Xiong, S. (2003) Hepatitis B virus

genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. Gastroenterology 125(1), 107-16.

Wolters, L.M., Hansen, B.E., Niesters, H.G., DeHertogh, D. and de Man, R.A. (2002) Viral dynamics during and after entecavir therapy in patients with chronic hepatitis B. J Hepatol 37(1), 137-44.

Wong, D.K., Cheung, A.M., O'Rourke, K., Naylor, C.D., Detsky, A.S. and Heathcote, J. (1993) Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis [see comments]. Ann Intern Med 119(4), 312-23.

Xiong, X., Flores, C., Yang, H., Toole, J.J. and Gibbs, C.S. (1998) Mutations in hepatitis B DNA polymerase asssociated with resistance to lamivudine do not confer resistance to adefovir in vitro. Hepatology 28, 1669-1673.

Yamamoto, T., Litwin, S., Zhou, T., Zhu, Y., Condreay, L., Furman, P. and Mason, W.S. (2002) Mutations of the woodchuck hepatitis virus polymerase gene that confer resistance to lamivudine and 2'-fluoro-5-methyl-beta-L-arabinofuranosyluracil. J Virol 76(3), 1213-23.

Yang, H., Westland, C., Xiong, S. and Delaney, W.E.t. (2004) In vitro antiviral susceptibility of full-length clinical hepatitis B virus isolates cloned with a novel expression vector. Antiviral Res 61(1), 27-36.

Yang, W. and Summers, J. (1995) Illegitimate replication of linear hepadnavirus DNA through nonhomologous recombination. J. Virol. 69, 4029-4036.

Yokosuka, O., Omata, M., Imazeki, F., Okuda, K. and Summers, J. (1985) Changes of hepatitis B virus DNA in liver and serum caused by recombinant leukocyte interferon treatment: analysis of intrahepatic replicative hepatitis B virus DNA. Hepatology 5(5), 728-34.

Yu, M. and Summers, J. (1994) Multiple functions of capsid protein phosphorylation in duck hepatitis B virus replication. J. Virol. 68, 4341-4348.

Zhang, X., Zoulim, F., Habersetzer, F., Xiong, S. and Trépo, C. (1996) Analysis of hepatitis B virus genotypes and pre-core region variability during interferon treatment of HBe antigen negative chronic hepatitis B. J. Med. Virol. 48, 8-16.

Zhou, T., Guo, J.T., Nunes, F.A., Molnar-Kimber, K.L., Wilson, J.M., Aldrich, C.E., Saputelli, J., Litwin, S., Condreay, L.D., Seeger, C. and Mason, W.S. (2000) Combination therapy with lamivudine and adenovirus causes transient suppression of chronic woodchuck hepatitis virus infections. J Virol 74(24), 11754-63.

Zhou, T., Saputelli, J., Aldrich, C.E., Deslauriers, M., Condreay, L.D. and Mason, W.S. (1999) Emergence of drug-resistant populations of woodchuck hepatitis virus in woodchucks treated with the antiviral nucleoside lamivudine [In Process Citation]. Antimicrob Agents Chemother 43(8), 1947-54.

Zoulim, F. (2001) Detection of hepatitis B virus resistance to antivirals. J Clin Virol 21(3), 243-53.

Zoulim, F. (2002) A preliminary benefit-risk assessment of Lamivudine for the treatment of chronic hepatitis B virus infection. Drug Saf 25(7), 497-510.

Zoulim, F. (2003) Treatment of pre- and post-liver transplantation HBV infection: Should we aim at combination therapy? Hepatology 38(6), 1353-5.

Zoulim, F., Dannaoui, E., Borel, C., Hantz, O., Lin, T.-S., Liu, S.-H., Trépo, C. and Cheng, Y.-C. (1996) 2',3'-dideoxy-b-L-5-fluorocytidine inhibits duck hepatitis B virus reverse transcription and suppresses viral DNA synthesis in hepatocytes, both in vitro and in vivo. Antimicrob. Agents Chemother. 40, 448-453.

Zoulim, F. and Seeger, C. (1994) Reverse transcription in hepatitis B viruses is primed by a tyrosine residue of the polymerase. J. Virol. 68, 6-13.