

# PERSPECTIVES ON THERAPY OF HEPATITIS B

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## I / THE EVOLVING CHALLENGES OF HEPATITIS B

### 1) INTRODUCTION

Despite a high rate of viral clearance by immunocompetent adult individuals, and the availability of efficient vaccines a large proportion of the world's population (400 million) is chronically infected with HBV. This is due to the fact that vertical transmission of HBV in neonates leads to a chronic infection in 90% of cases. The pathology is immune-mediated. The coexistence of repeated cycles of HBV replication and immune lysis of infected hepatocytes is associated with fibrosis, cirrhosis, and hepatocellular carcinoma. 250,000 deaths each year are still resulting from hepatitis B.

Current strategies for treating hepatitis B have focused on clearance of active HBV infection through suppression of viral replication. The efficacies of these treatments have been determined by monitoring the levels of HBV DNA in the serum, serum ALT levels, loss of viral antigens (HbeAg and HBsAg), seroconversion (HBeAb and HBsAb), and ultimately by improvements in liver histology. Interferon- $\alpha$  (IFN- $\alpha$ ) and the nucleoside analogs (lamivudine and adefovir) have proven their effectiveness by these clinical markers and are currently approved for treatment of chronic hepatitis B. Sustained virological response rate however ranges between 15 and 40 % (1). The actual rate of sustained response in non selected patients is supposed to be lower. Following the introduction of interferon acting mainly through immune potentiation, the development of new antivirals that inhibit the viral polymerase has provided new hope in the therapy of chronic hepatitis B. However due to the slow kinetics of viral clearance, long-term treatment is mandatory and unfortunately, the spontaneous genetic heterogeneity of the virus prompted drug resistant mutants selected by the treatment. It is therefore alike for HIV critical to develop new antiviral strategies to better combat wild type and mutant HBV infections.

### 2) MOLECULAR TARGETS OF THE HBV REPLICATION CYCLE

HBV belongs to the hepadnaviridae family which includes fish, birds, mammals and monkey viruses. Experiments performed in animal models of HBV infection have contributed to the molecular characterization of the replication cycle of HBV in infected hepatocytes. This led to the identification of the first viral targets for antiviral therapy focusing on the reverse transcription step (2). The key role of the viral covalently closed circular DNA (CCC DNA) as a template of viral transcription and for the maintenance of viral infection at the level of a single hepatocyte was well documented. There are still important issues that remain unresolved, such as the characterization of the cellular receptor required for viral entry (3).

Therefore, two main non-exclusive strategies can be envisioned to eradicate viral infection : i) inhibition of viral replication using different approaches including neutralization of viral infection by inhibiting the virus-cell interaction with anti-envelope antibodies, cytokine and/or anti-sense inhibition of viral transcription and/or viral gene expression, inhibition of packaging with peptides, inhibition of the RNA or DNA dependent DNA polymerase activity; ii) enhancement of the specific immune response, using cytokines like interferon alpha,

gamma, TNF alpha, interleukine 2 and 12, or viral recombinant protein or plasmid DNA based vaccine approach.

Another important aspect of chronic HBV infection is the integration of the viral genome in the host genome. By contrast to retroviruses, this phenomenon is not required for viral replication but is one of the major factor involved in virus induced hepatocarcinogenesis (4). This process does represent another potential target for which novel approaches are required to prevent the development of liver cancer in chronically infected patients with minimal viral replication but integrated viral genome sequences which most likely turn on the oncogenic process.

### 3) VIRAL KINETICS AND IMMUNE CLEARENCE

Mechanisms of viral clearance are thought to involve professional CD8+ cells exhibiting cytolytic activity against infected hepatocytes as well as the production of antiviral cytokines, cell turn over with the replacement of infected cells by new hepatocytes, viral neutralization by anti-envelope antibodies produced by B-cells. Specific CD4 cells may also contribute to viral clearance by regulating the TH1/TH2 balance. The exact sequence of molecular events during viral clearance remains unclear. Viral clearance may occur in massively infected animals without significant liver damage (5). This provides the basis for using a combination of nucleoside analogs and cytokines to hasten viral clearance.

This complex host immune response interaction with infected cells explains 3 phases of the natural history of chronic HBV infection. The fact that HBV replication does not induce a cytopathic effect and that viral CCC DNA may have a long-half life in infected hepatocytes, are key determinant in viral persistence and selection of resistant mutants.

## II / FUTURE MOLECULAR APPROACHES

### POLYMERASE

So far the most active compounds registered or in development have focused the HBV multifunctional viral polymerase that is involved throughout the replication reaction. HBV polymerase has both RNA- and DNA-dependent polymerase activities as well as an RNase H activity that degrades pre-genomic RNA during reverse transcription (6). Although HBV polymerase activity can be measured in several *in vitro* systems, the type of detailed structural and biochemical information available for HIV reverse transcriptase is not available for HBV polymerase due to the inability to express large quantities of functional, non-capsid associated polymerase in heterologous systems. Nevertheless, targeting the HBV polymerase has been achieved. The availability of stable cell lines capable of producing HBV particles has been critical for screening nucleoside analogs for antiviral activity.

The main issue for lamivudine whether a sustained response can be achieved before the emergence of drug resistant mutants, must still be addressed with the newer treatments. In this regard, combinations of different nucleoside analogs may be necessary. Adefovir Dipivoxil was shown to be active in patients with lamivudine resistant HBV (7) and therefore has potential for use in combination therapy with lamivudine. However, FTC and L-FMAU are cross-resistant to lamivudine resistant HBV mutants *in vitro*, so their utility in pre-treated patients or in combination with lamivudine may be limited. Whether the polymerase will have the plasticity of the HIV reverse transcriptase in tolerating additional mutations to acquire resistance to multiple drugs remains to be determined, but is suggested by the sequential selection of famciclovir and lamivudine resistant strains.

### 2) CORE

The core gene encodes the viral capsid protein (HBcAg). The role of core protein in viral assembly is complex and involves interactions that direct envelopment and secretion. Given the central role that the core protein plays in virus maturation, agents that interfere with nucleocapsid assembly may possess antiviral activity, as well as molecules affecting its phosphorylation. Although no small molecules are presently known

to inhibit capsid assembly or maturation, the existence of dominant negative core mutants that inhibit viral replication (8) and of peptides capable of blocking the association between core particle and surface antigens and inhibiting virus production (9) indicates that disruption of assembly does affect viral replication. Furthermore, AT-61, a small molecules inhibitor of HBV replication from Avid Therapeutics (10) reduced the amount of core particles containing pre-genomic RNA suggesting that assembly was affected, although the exact mechanism remains unknown. Thus, targeting core protein assembly or maturation may have potential as a therapeutic modality.

### 3) ENVELOPE PROTEINS

Infectious HBV particles contain three forms of envelope protein termed L, M, and S. These are produced from a single ORF through alternative transcription and translation initiation sites. All three forms of envelope protein are inserted into the endoplasmic reticulum membrane and become glycosylated as they are transported through the secretory pathway. They are involved in binding to the cellular receptor for viral uptake. The identity of the receptor for HBV remains elusive but carboxypeptidase D has been identified as a receptor for the duck hepatitis B virus (DHBV). Additional, as yet unidentified, factors are needed after the initial binding event to allow infection to occur and these may be functionally analogous to the co-receptors identified for HIV. The identification of the human HBV receptor should be greatly facilitated by the establishment of an *in vitro* infection system (11) which will allow HBV particle entry to be targeted. The prospects are poor for therapies that target HBV particle entry. The envelope proteins are post-translationally modified, and inhibitors of  $\alpha$ -glucosidase I, a cellular enzyme involved in oligosaccharide processing, have been found to inhibit the secretion of M protein but no S or L proteins. Egress of the envelope protein, in addition to ingress via receptor binding, may therefore be a target for antiviral therapy. In the case, inhibition of the viral process is affected through inhibition of a cellular target. The resistance profile of the  $\alpha$ -glucosidase inhibitors will clearly differ from the polymerase inhibitors and will enable these two classes of inhibitors to be used in combination. However, toxicity arising from inhibiting cellular processes is an issue that will need to be addressed.

### 4) ANTISENSE MOLECULES AND RIBOZYMES

Transcription and translation of HBV DNA and HBV RNA can be prevented by antisense molecules or ribozymes that are complementary to the DNA or RNA templates. The advantages of this approach is that specific targets can be selected. In addition, the risk of drug-resistant mutants can be reduced by targeting multiple sites in the viral DNA or RNA or by targeting regulatory sequences that would not tolerate mutations. *In vitro* studies have confirmed that this approach is feasible. The major impediments to clinical use include rapid degradation of the antisense molecules by nucleases *in vivo*, lack of an efficient delivery system into the target cells and hindrance of access to target DNA or RNA sequences by secondary structure. Several pilot studies have demonstrated the feasibility of delivery of antisense molecules to DHBV-infected ducks and the efficacy of these molecules in inhibiting DHBV replication and viral protein expression (12,13). Ribozymes act enzymatically and have the theoretical advantage that a single ribozyme can catalyze the irreversible inactivation of multiple substrate molecules. Several groups have demonstrated the *in vitro* efficacy of ribozymes to cleave target HBV sequences. Pilot studies in mice showed that nuclease-resistant synthetic ribozymes can be delivered to the liver in sufficient concentrations for antiviral effect (14). Phase III clinical trials are being planned.

## III / COMBINING STRATEGIES

### 1) IMMUNOTHERAPY

The host immune system is central to controlling HBV infection and to the pathogenesis of the disease. Individuals who clear HBV infection have a vigorous polyclonal class I-restricted cytotoxic T lymphocyte (CTL) and class II-restricted CD4+ T-helper response to HBV proteins. In contrast, the CTL responses in chronically infected patients are difficult to detect and narrowly focused. Extensive work with the transgenic mouse model of HBV has shown that CTLs can abolish HBV expression and replication by a noncytotoxic mechanism (15).

Clearance of HBV DNA in the liver of infected chimpanzees during an acute infection also occurs by a noncytopathic mechanism. Clearance of virus requires that nuclear cccDNA is lost from the regenerating hepatocytes and that they are protected from reinfection. Understanding the components of the immune system involved in these processes will allow the rational development of immunomodulatory agents for treatment.

## 2) New approaches in animal models

It was shown in DHBV infected primary duck hepatocytes that recombinant duck interferon gamma inhibits DHBV replication in a dose-dependent fashion as well as the synthesis of progeny CCC DNA by amplification but does not affect the initial formation of CCC DNA. Another interesting finding was that treatment with duck interferon alpha resulted in a different antiviral effect with a depletion of pregenomic RNA containing nucleocapsids (16). Since most chronic HBV carriers do not respond to interferon therapy, it has been hypothesized that the limited efficacy of interferon may rely at least in part to a low expression of IFN receptor in the liver. Since the asialoglycoprotein (ASGP) receptor is highly expressed in the liver, the ASGP receptor binding domain was generated within a N-glycosylated human interferon beta molecule. This modified IFN beta molecule exhibited a greater anti-HBV effect in vitro in tissue culture compared to its conventional counterparts, ie IFN alpha and beta. This was also supported by a stronger induction of the 2',5' oligo-adenylate synthetase. Moreover, this modified IFN beta was shown to significantly inhibit HBV replication in vivo, in a nude mouse model, in contrast to conventional IFN beta. These results indicate that directing IFN to ASGP receptor may facilitate its targeting to the infected liver and enhance the antiviral effect of IFN. Due to their many clinical implications, these findings deserve rapid confirmation in clinical trials.

Another interesting approach to stimulate the anti-HBV immune response would be to use therapeutic vaccines as discussed by Pol et al in this symposium. Very interesting findings came from studies performed in ducks that were chronically infected with DHBV in whom DNA based vaccination with a pre-S envelope protein encoding plasmid induced a strong humoral response against the pre-S antigen and inhibited dramatically viral replication within the liver (17). These important results deserve confirmation in animal models and in clinical trials before designing new protocols for the combination of nucleoside analogs and immune manipulation.

## 3) THE GLOBAL APPROACH : Multiple combination options

With the problem of the persistence of infected cells and the selection of drug resistant mutants, the concept of combination therapy of chronic hepatitis B virus infection is becoming a critical issue (19). The combination of polymerase inhibitors is one of the potential approaches. To obtain a synergistic antiviral effect, a combination of nucleoside analogs exhibiting different mode of actions (anti-priming, anti-elongation, purine analogs, pyrimidine analogs) is expected to give the best chances of obtaining a synergistic antiviral effect. The main nucleoside analogs that have been evaluated in experimental models or in clinical trials may represent promising candidates for combination therapy (20). A few studies have shown that the combination of lamivudine with penciclovir and / or adefovir may inhibit synergistically DHBV replication in primary duck hepatocyte cultures. This should in turn decrease statistically the risk of selection of polymerase mutants. The combination of compounds with different patterns of viral resistance will also be required to further decrease the risk of selecting multiple drug resistant mutants.

When they will become available it is clear that targeting different steps of the viral cycle and morphogenesis will be most likely synergistic. Again the examples in HIV therapy do suggest it. The combination of nucleoside analogs to inhibit viral production with immune modulatory approaches to eradicate the residual infected hepatocytes that may support the replication of escape mutants should now be evaluated in animal models of hepadnavirus infection to determine its capacity to clear viral infection or only control its replication. Ultimately for biological but also economical reason the control of hepatitis B will most likely rely on synergistic strategies involving cheap therapeutic vaccine which developing countries could afford.

## **IV / PREVENTING HCC HEPATOCELLULAR CARCINOMA**

The main complication and cause of death of chronic HBV infection is HCC. Even inhibition of HBV replication if achieved at a later stage may not suppress this risk as this was documented in the wood chuck model with potent antivirals (21). Preliminary studies have documented a potential benefit of IFN in some reports but not in others.

Remarkably again experimental studies in transgenic mice recapitulating HBV induced hepatocarcinogenesis have documented the possibility of reducing HCC by early and dose adjusted IFN regimen (18). Additional studies in relevant models may allow to target the different genes involved. Likewise gene therapy may also be used in this regard in the future.

## REFERENCES

1. 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.
2. Summers, J. and Mason, W.S. (1982) Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell* 29, 403-415.
3. Urban, S., Schwarz, C., Marx, U.C., Zentgraf, H., Schaller, H. and Multhaupt, G. (2000) Receptor recognition by a hepatitis B virus reveals a novel mode of high affinity virus-receptor interaction. *Embo J* 19(6), 1217-27.
4. Mason, W.S. and Taylor, J.M. (1989) Experimental systems for the study of hepadnavirus and hepatitis delta virus infections. *Hepatology* 9, 635-645.
5. 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. *J Virol* 74(3), 1495-505.
6. Radziwill G., Tucker W., Schaller H. Mutational analysis of the hepatitis B virus P gene product: domain structure and RNase H activity. *J Virol* 1990; 64:613-620.
7. Perrillo R., Schiff E., Yoshida E., Statler A., Hirsch K., Wright T., Gutfreund K., Lamy P., Murray A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* 2000; 32:129-134.
8. Scaglioni P.P., Melegari M., Wands J.R. Characterization of hepatitis B virus core mutants that inhibit viral replication. *Virology* 1994; 205:112-120.
9. Dyson M.R., Murray K. Selection of peptide inhibitors of interactions involved in complex protein assemblies: Association of the core and surface antigens of hepatitis B virus. *Proc Natl Acad Sci USA* 1995; 92:2194-2198.
10. King R.W., Ladner S.K., Miller T.J., Zaifert K., Perni R.B., Conway S.C., Otto J.J. Inhibition of human hepatitis B virus replication by AT-61, a phenylpropenamide.
11. P. Gripon et al. Successful HBV infection of a new highly differentiated human
12. hepatoma cell line. *Molecular Biology of Hepatitis B Viruses*, Amherst, Massachusetts, USA, July 29-August 2, 2001.
13. Von Weizsacker F, Wieland S, Kock J, Offensperger WB, Offensperger S, Moradpour D, Blum HE. Gene therapy for chronic viral hepatitis: ribozymes, antisense oligonucleotides and dominant negative mutants. *Hepatology* 1997; 26:251-5.
14. Robaczewska M, Gueret S, Remy JS, Chemin I, Offensperger WB, Chevallier M, Behr JP, Podhajska A, Blum HE, Trepo C, Cova L. Inhibition of hepadnaviral replication by polyethylene glycol-based intravenous delivery of antisense phosphodiester oligodeoxynucleotides to the liver. *Gene Therapy*, 2001; 8:874-881.
15. Morrissey DV, Johnson DA, Macejak DG, Pavco PA, Mockler V, Keen B, Blatt LM. Inhibition of HBV protein synthesis by nuclease resistant ribozymes [abstract]. *Hepatology* 2000; 32 :459A.
16. Guidotti L.G., Ishikawa T., Hobbs M.V., Matzke B., Schreiber R., Chisari F.V. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 1996; 4 :25-36.
17. 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.
18. Rollier, C., Sunyach, C., Barraud, L., Madani, N., Jamard, C., Trepo, C. and Cova, L. (1999) Protective and therapeutic effect of DNA-based immunization against hepadnavirus large envelope protein. *Gastroenterology* 116(3), 658-65.
19. Merle P, Chevallier M, Levy R, Maisonnas M, Terradillos O, Si Ahmed SN, Trepo C, Buendia MA, Vitvitski-Trepo L. Continuous interferon-alpha therapy decreases oncogenesis in a mouse transgenic hepatocellular carcinoma model recapitulating the outcome of woodchuck hepatitis B like virus chronic infection. *J Hepatol*, 2001, 34:562-569.
20. Zoulim F. 2001. Evaluation of novel antiviral strategies targeting wild type and drug resistant mutants in experimental models. *Antiviral Chemistry and Chemotherapy* ; 12 (suppl 1) : 131-142.
21. Seignères B, Pichoud C, Martin P, Furman P, Trépo C, Zoulim F. Inhibitory activity of dioxolane purine analogs on wild type and lamivudine resistant mutants of hepadnaviruses. *Hepatology* ; 2002: in press.
22. Le Guerhier F, Pichoud C, Jamard C, Guerret S, Chevallier M, Peyrol S, Hantz O,

23. King I, Trépo C, Cheng YC, Zoulim F. 2001. Antiviral Activity of beta-L-2',3'-Dideoxy-2',3'-Didehydro-5- Fluorocytidine in Woodchucks Chronically Infected with Woodchuck Hepatitis Virus. *Antimicrob. Agents Chemother.* 45 : 1065-1077.