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EDITORIAL

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Recent advances in hepatitis B virus research: A German point of view

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Abstract

More than 30 years after the discovery of human hepatitis B virus (HBV) this virus remains to be one of the major global health problems. In infected adolescents or adults, 5%-10% will lead to a chronic carrier state, whereas in infected neonates up to 90% develop chronicity. It is estimated that about 370 million people are chronic carriers of HBV worldwide. In many regions of the world, chronic HBV infection is still the major cause of liver cirrhosis and hepatocellular carcinoma. During the last 30 years, many steps of the viral life cycle have been unravelled, mainly due to cloning, sequencing and expression of the genomic DNA extracted from HBV virions. This has lead to the development of a safe and efficient vaccine and sensitive tests for HBV surface protein (HBsAg) allowing reliable diagnosis and screening of blood products. More recently, a growing number of reverse transcriptase inhibitors have been developed. However, together with these improvements new deficiencies in prevention and cure of HBV infections are becoming apparent. Although HBV is a DNA virus, it is highly variable under immunity or drug induced selection pressure, resulting in vaccine-related escape mutants and drug resistance. To overcome these challenging problems new antivirals and optimised vaccines have to be developed.

Key words: Hepatitis B virus; Molecular biology; Viral life cycle; Animal system; Antiviral therapy

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The discovery of the Australian antigen by Baruch S. Blumberg, which is now known as "hepatitis B virus surface antigen" (HBsAg), in the late 1960's opened the field of HBV research towards an understanding of an ancient scourge. During the following decades, many research groups all over the world worked successfully to unravel the life cycle of HBV. In Germany, the virologists Heinz Schaller, Peter H. Hofschneider, and Reiner Thomssen initiated HBV research and provided a profound impact on our understanding of the molecular biology of this virus. Among the clinicians; the names of Karl-Hermann Meyer zum Büschenfelde and Wolfgang Gerok are to be mentioned as founders of hepatitis B research. Moreover, in their labs, but also in other groups, students and scientists became inspired to work on this fascinating virus. Since then, an active and growing community of HBV virologists has been established in Germany. In this highlight topic a collection of reviews written by those scientists and clinicians is presented. The articles cover nearly all fields of hepadnaviral research, from attachment and entry to genome replication, maturation, pathology, animal models and antiviral therapy. The aim of this collection is to bring together recent findings in basic virological and clinical HBV research in order to provide a valuable reference for both hospital hepatologists and basic scientists.

Hepatitis B virus is the prototype member of the family *hepadnaviridae* that can be divided into the *orthohepadnaviruses* of mammals and the *avihepadnaviruses* of birds. To date, the *orthohepadnaviruses* are found only in humans and primates and in a special family of the *sciuridae*, namely North-American woodchucks and some squirrel species. Primate HBV were found in old world primates like chimpanzees, gorillas, gibbons, orangutans, and in one new world primate, the woolly monkey. In the review "Taxonomy and genotypes of Hepatitis B Virus", Stephan Schaefer reports that HBV sequences from old world primates are closely related and cluster in monophyletic groups, while the HBV sequence isolated from woolly monkeys (WMHBV) is more divergent[1]. Human HBV is further subdivided into several genotypes. To date, 8 genotypes (called A-H) that differ by definition in at least 8% of their complete genome have been found. The genotypes can be further divided into 24 sub-genotypes that differ by at least 4% from each other. Interestingly, no sub-genotypes have been described for genotype E and G. The fact that genotype E is common in West Africa, but absent in Americans of African origin from Venezuela and Brazil has led to the speculation of a recent genesis of genotype E. The geo-

graphic origin of genotype G is less clear, however a high prevalence of this genotype has been described in Mexico. Both, the genotypes and sub-genotypes show distinct virological and epidemiological properties. As an example, liver cirrhosis was detected in a greater number of patients chronically infected with HBV genotype C than genotype B in Japan. The same was also detected for the prevalence of hepatocellular carcinoma in these patients. Interestingly, detailed analysis of HBV genomes revealed recombination events between different genotypes. Hybrids between genotype B and C, A and D are very common in certain regions and distinct intergenomic recombination breakpoint hotspots were detected (e.g. the preS1/S2 region and the 3'-end of the surface gene). B/C recombinants occur in Japan and C/D recombinants in Tibet. However, the mechanisms underlying these potential recombination events are still unknown. In contrast to HIV or RNA viruses, recombination during replication is unlikely, because reverse transcription of only one RNA genome occurs within the capsid. Thus, it seems also possible that the described changes are the result of an adaptation to distinct genetic disposition in different human populations. Taking into consideration that a chronic HBV carrier can produce up to 10^{13} virions per day together with a high error rate of HBV reverse transcriptase, the HBV quasispecies can substitute every nucleotide of the small 3.2 kb genome within one patient every day. This favours very fast adaptation of the virus to a changing environment, and may result in modular adaptations within distinct segments of the genome.

Soon after the discovery of hepatitis B virus, it was shown that not only humans but also chimpanzees may test positive for this agent. Furthermore, artificial infection of chimpanzees, but also other apes like gibbons and orangutans with serum from HBV-infected patients induces acute and chronic infections related to human disease. Nevertheless, a strong species specificity of hepatitis B virus was observed, since it was not possible to induce HBV-infection in other mammals. Since then, many efforts have been made to establish a small non-primate animal model for HBV infection *in vivo* as described in the review "Viral and cellular determinants involved in hepadnaviral entry" in this topic highlight[2]. The finding that *Tupaia belangeri*, a small non-primate mammal from Southeast-Asia is susceptible to HBV and HDV raises doubt in the strict host specificity of HBV. Besides the *in vivo* infection, which is usually self-limiting and does not lead to chronic infection, the use of primary Tupaia hepatocyte cultures for *in vitro* infection has greatly improved the field. For many years, primary human hepatocyte cultures, obtained after surgical liver resection were the only possibilities to study infectivity of HBV *in vitro*. However, working with these scarcely available cell cultures is not easy and optimal HBV infection is highly dependent on artificial additives like dimethylsulfoxide (DMSO) and polyethyleneglycol (PEG) for optimal infection. Furthermore, susceptibility of these cultures varies strongly. Primary hepatocyte cultures from *Tupaia belangeri* have overcome these limitations both in availability and susceptibility. Furthermore, the infection is possible without the addition of DMSO and PEG. Most established hepatoma cell lines allow HBV production, but only after transfection of HBV DNA. Nevertheless, in 2001, a new hepatoma cell line called HepaRG was established that could be infected with HBV. However this feature is achieved only after prolonged cultivation in medium containing DMSO and other additives.

Experiments 20 years ago using synthetic peptides gave the first hint on the importance of the preS1 domain for viral infectivity. Later it was confirmed that the first 77 aminoacids of this domain are necessary for HBV infectivity, together with the N-terminal myristoylation at Glycin-2. Combining these important features, it turned out that these myristoylated preS1-peptides inhibited HBV infection at nanomolar concentrations *in vitro*. The exact role of myristoylation during the entry process of HBV is still not clear. It might enhance receptor recognition by insertion of the acyl chain into membranes of the receptor-complex. Interestingly, a so called myristoyl-switch is a well known element of viral entry mechanism used by non-enveloped viruses, like picornaviruses and reoviruses. Using a set of different myristoylated preS1-peptides the preS1 sequence responsible for this inhibition was further narrowed down to 10 aminoacids (NPLGFFPDHQ) in position 9-18 of preS1. Even single point mutations within this region abolish the inhibitory potential of preS1 peptides and when introduced into the viral surface proteins, also destroy infectivity of the virus completely.

The MHBs of HBV seem to be dispensable for infectivity of HBV and HDV. However, antibodies raised against the preS2 region that is an integral part of MHBs and LHBs could inhibit infection both *in vivo* and *in vitro*. Controversies exist about a role of a putative translocative motive (TLM) within the carboxyterminal part of preS2 and its importance for infectivity of HBV. However, no function of this sequence has been attributed to infection with HDV that uses HBV surface protein for infection.

The S-domain contributes to the main part of HBV surface proteins, but from the data obtained by use of myristoylated preS1 peptides, we know that the S-domain is not involved in initial binding to target cells. Nevertheless, antibodies raised against this domain are neutralising and contribute to protection against HBV infection in most cases. However, antibodies against the so-called antigenic domain (residues 100-160 that are also generated by the current S-containing vaccines) may not protect against naturally occurring escape-mutants that are frequently selected, e.g. by antiviral treatment. Since we now are aware that these antibodies will not counteract the binding of the virus to its target cells, the preS1 domain containing the minimal interaction site ought to be included into the vaccine. An unsolved problem is the identity of the still undetected high affinity receptor for HBV. An update of the still growing list of potential receptor candidates is listed in this reviews series, but none of them has ever been shown to be relevant for HBV infection.

Inspired by the presumption that the infection process of all hepadnaviruses may follow similar pathways, many labs used the more easy accessible duck hepatitis B virus (DHBV) infection system to study hepadnaviral entry *in vitro*. Although also restricted to primary hepatocyte cultures, this system is, compared to primary human hepatocyte cultures, relatively easy and accessible. Like in

HBV, the preS-domain of large surface protein of DHBV contributes to infection together with its N-terminal myristoylation. Furthermore, the preS-domain binds to a 180 kDa membrane protein and this binding can be inhibited by neutralizing preS-antibodies. Further work identified the gp180/p170 protein as carboxypeptidase D (CPD), a transmembrane protein with enzymatic activity. While substantial evidence supports the essential role of CPD for the DHBV infection process, expression of CPD in non-susceptible cells did not make these cells susceptible towards DHBV. Therefore, the first attachment partners for DHBV are still unknown. Furthermore, this molecule seems to be restricted to *avihepadnaviruses*, since the human homolog of CPD does not contribute to the HBV infection process. Therefore we have to be aware of the possible differences of entry mechanism of *avi-* and *orthohepadnaviruses*. Nevertheless with easy accessible *in vitro* infection systems for HBV available (primary Tupaia hepatocytes and the HepaRG cell line) it should now be possible to characterize cellular attachment factors and entry receptors for HBV.

After uptake, the viral genome has to be transported through the cytoplasm to the nucleus where active transcription can take place. As discussed by Kann *et al*[3]. evaluation of the transport modes of HBV from the plasma membrane to the nucleus is not easy since at least the early steps of attachment and entry are very species specific and usually need primary human or Tupaia hepatocytes. After uptake of the virus in a yet unknown compartment, the viral envelope proteins mediate fusion of viral and cellular membranes that results in delivery of the viral capsid into the cytoplasm. The capsid, harbouring the viral DNA, covalently linked to the viral polymerase must then be transported into the nucleus of the host cell. To study HBV capsid transport without an infection, isolated HBV capsids have to be either analysed within permeabilized cells or microinjected into *Xenopus laevis* oocytes. Both systems are very artificial and time-consuming. However, in the review of Kann *et al*, an easy *in vitro* system is described that allows analysis of intracellular transport after artificial entry of the capsids. The authors replaced the viral surface proteins of the virus by lipids, normally used for protein transfection. The lipids form an artificial membrane that allows fusion with the cellular plasma membrane and release of the viral capsid into the living cell. In fact, lipofection of HBV capsids isolated from virions allows "infection" of cells that are non-susceptible to the whole, complete virus. First, the core particles are transported *via* microtubuli towards the microtubule-organising centre, located at the perinuclear region of the cell. The viral capsid, containing a nuclear localisation signal interacts with the adaptor proteins importin alpha and beta. Importin beta facilitates contact with the nuclear pore and translocation into the nuclear pore. Interestingly, despite complete translocation, the core particles seem to be stuck at the end of their voyage through the nuclear pore, in a structure called "nuclear basket". Within the nuclear basket, the breakdown of the capsid and the release of the viral DNA into the nucleus should be facilitated. However, many questions are still unanswered, e.g. what determines the arrest of the core particle within the nuclear basket and genome release?

Once within the nucleus of the infected cells, the viral HBV genome is converted into a stable form that allows continuous production of progeny virus and is not lost during cell division. Jürgen Beck and Michael Nassal, give an overview of the hepadnaviral genome replication, starting from the conversion of the incoming viral genome with its circular, but only partially double stranded (rcDNA) to the very stable covalently closed circular DNA (cccDNA)[4]. The rc-DNA form of the HBV genome contains diverse modifications (e.g. the polymerase-protein covalently linked to the 5'end of the (-)-DNA strand) that have to be removed before generation of cccDNA that can serve as a matrix for proper transcription. Experimentally, this is difficult to investigate, since detection of cccDNA in the presence of high amounts of rcDNA is not trivial. Furthermore, cccDNA is present only in low amounts in infected hepatocytes (from 10-50 genomes per cells). Nevertheless, the episomal cccDNA is very stable (half-life > 30-60 d for DHBV infected ducks) and can therefore even persist during effective antiviral therapy. A detailed understanding of rc- to cccDNA conversion and its inhibition would therefore be desirable. The HBV cccDNA serves as the template for all viral RNAs that are transcribed by cellular RNA polymerase II. Interestingly, the reverse transcription step of HBV requires the specific packaging of the pregenomic HBV RNA together with the viral polymerase (containing reverse transcriptase, DNA polymerase and primase domains) into newly formed capsids. Therefore, the authors describe the newly formed capsid as a "dynamic replication machine" that puts the compartmentalization of genome amplification, known from other viruses, to its extreme. Besides the capsid, viral reverse transcriptase and pregenomic RNA, additional factors are essential for hepadnaviral replication. New methods that use *in vitro* reconstitution of purified components are beginning to reveal that cellular chaperones (e.g. heat shock proteins, hsps) are essential factors for viral polymerase-protein activation. Cell-free reconstitution systems will allow scientists to systematically study the factors necessary for hepadnaviral replication.

The review by Volker Bruss summarizes our current knowledge about hepatitis B virus morphogenesis[5]. Envelopment of mature core particles depends on the presence of HBV surface proteins that are synthesized at the endoplasmatic reticulum (ER). HBV envelope proteins contain three related co-carboxyterminal surface proteins. Common to all three proteins is the 226 aminoacids long small surface protein (SHBs). Aminoterminal addition of the 55 aminoacid long preS2-domain results in the middle surface protein (MHBs), while further aminoterminal addition of the preS1-domain (109 or 118 aminoacids, depending on the genotype) are found in large surface protein (LHBs). During synthesis at the ER, the SHBs builds a conformation that results in exposure of aminoacids around 99 to 169 to the ER lumen resulting in N-glycosylation of half of the S proteins at asparagine (asn) 146. After budding, this loop is located at the surface of virions and subviral particles and carries the major conformational epitope of the HBV surface proteins. The M-Protein, in addition to the

S-domain, is always N-glycosylated at asn-4 in preS2 and also present on the viral surface. The preS2-domain is also O-glycosylated at threonine (thr) 37, but interestingly only in genotypes B-H, since genotype A lacks thr-37. However, the overall function of this O-glycosylation for the viral life cycle is still unknown. Moreover, the function of the M-protein by itself is still not clear. Absence of the M-protein does neither suppress virion formation nor impede viral infectivity. However, the M-protein seems to have an evolutionary advantage for the virus, since it is conserved through all *orthohepadnaviruses*. The preS2-domain is a further integral building block of the large surface antigen (LHBs) by further aminoterminal addition of the preS1-domain to the preS2-domain. The preS1-domain has a dual function: the aminoterminal domain is necessary for attachment and entry of the virus, while the carboxyterminus together with aminoterminal part of preS2 is used for envelopment. Interestingly, the preS-domains of the LHBs are cytosolic during translation but half of the preS chains of LHBs are believed to translocate after translation. How the preS domains cross the membrane is still unknown, however cytosolic and ER-specific chaperones are believed to be involved in this process. In addition, the preS1-domain is myristoylated at Glycin-2, a modification that is not necessary for viral morphogenesis, but important for efficient viral infection. Even without involvement of the core particle, the surface proteins can bud from a post-ER, pre-Golgi compartment and build subviral particles that do not contain a viral capsid or DNA and are therefore non-infectious. In the case of HBV, they are built in the form of spheres and filaments and can reach concentrations that are 10 000-fold higher than the virions. Interestingly, the L-protein cannot build particles by itself, but needs S or M protein for proper segregation. Moreover, the LHBs can induce a dose-dependent inhibition of particle release even in the presence of SHBs. A massive storage of HBV envelope proteins in turn can lead to massive cell stress causing cell death or cancer. The significance of the secretion inhibition function of LHBs for the viral life cycle is still not clear. However, it seems to be dependent on the myristoylation of preS1 domain of LHBs since blocking of LHBs myristoylation abolishes the storage phenomenon of LHBs.

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors world-wide. Behind lung and stomach cancer, HCC is the one of the leading cause of cancer death. In the review by Joachim Lupberger and Eberhard Hildt, titled "HBV Induced Oncogenesis", the authors discuss the relation of chronic HBV infections and HCC and describe the epidemiology of HBV-associated HCC[6]. Epidemiological data state that chronic HBV carriers have a more than 25 times higher risk of developing HCC, while the molecular mechanism underlying the development of HBV-associated HCC are still not clear. The authors distinguish between direct effects and indirect effects caused by the integration of HBV-DNA into the host genome. In contrast to retroviruses, integration of viral HBV-DNA is not necessary for the viral replication. However, almost all HBV-associated HCCs contain integrated HBV DNA. The integrated viral genomes are rearranged or contain deletions, and cannot lead to viable progeny. However, they may exert an effect on key regulators of the cell cycle. Of special interest in this respect is the frequent integration of woodchuck hepatitis B virus into the N-myc2 gene of the host cells of North-American woodchucks in WHV-related HCC. In HBV-associated HCC however, site-specific integration of HBV genome is a rare event. One indirect effect of integrated HBV-DNA is transcription of HBV open reading frames that remain conserved even after integration. Of special interest is the HBx protein, a small polypeptide that is produced at very low levels in HBV infected hepatocytes. A large number of possible functions have been ascribed to this still enigmatic protein. Initially described as a transcriptional activator, the authors discuss interference of HBx with different signal transduction cascades. Furthermore, HBx was found to interfere with DNA repair and might therefore account for an increase of critical cellular mutations that might increase the risk of developing HCC. Besides the HBx, a special form of the HBV surface protein was found in HBV-infected hepatocytes of HCC patients. The middle surface protein (MHBs) was found to be C-terminally deleted in its S-domain (MHBst) that in turn results in an altered topology of the aminoterminal preS2 domain. While the preS2-domain of wildtype MHBs is located in the lumen of the endoplasmatic reticulum (ER) and is glycosylated at asparagine 4, the preS2-domain of the truncated form is in the cytoplasm. This results in interaction of preS2 with protein kinase C (PKC) and permanent activation of Raf/MEK/ERK signal transduction cascades that might exert a tumor promoter-like function. In line with this, transgenic mice expressing MHBst develop liver tumors. However, a more detailed understanding of the molecular mechanism in HBV-associated HCC development is needed.

In general the infection with HBV can lead to a wide spectrum of clinical manifestations, e.g. self-limited acute or fulminant hepatitis, asymptomatic infection, or chronic hepatitis with progression to liver cirrhosis that can lead to hepatocellular carcinoma (HCC). In their review, Baumert *et al*[7] focus on the impact of virus-host interactions for the pathogenesis of HBV infection and associated liver disease. Both, viral factors and host immune response are discussed. One of the major viral factors are certain HBV mutants that are associated with distinct clinical manifestations, altering the natural course of the infection and confer resistance to antiviral agents, e.g. inhibitors of HBV reverse transcriptase. The authors focus mainly on pre-core (pre-C) stop codon mutations resulting in loss of hepatitis B e antigen (HBeAg) and core-promoter mutations that enhance viral replication. The pre-C stop codon mutation is clinically recognized mainly in patients with chronic and fulminant hepatitis but is also detected in asymptomatic HBV carriers or self-limiting hepatitis. In all these patients, HBeAg, a soluble and secreted form of the core-protein cannot be synthesized any more, despite active viral replication. Therefore the authors argue that HBeAg may play an important role for the interaction of the virus with the host immune system, e.g. HBeAg might have an immunomodulatory function. In line with this, HBeAg might predispose neonates born to HBV-

infected mothers to develop persistent HBV infection by establishing T-cell tolerance to HBeAg and HBcAg *in utero*. Core-promoter mutations are mainly found in patients with an aggressive course of disease like fulminant hepatitis B. Those mutations result in a viral phenotype that shows enhanced viral replication in cell culture and might alter viral kinetics and influence cellular immune response *in vivo*. This might result in more severe liver injuries and possibly fulminant hepatitis. Despite the effect of viral variants, the host immune response is the key player in the onset of liver disease. The authors describe that HBV does not induce strong immune response during the early onset of infection. At the onset of HBV clearance an influx of T cells into the liver occurs, but this is not typically associated with strong liver disease, suggesting non-cytopathic mechanisms for viral clearance that is CD8+ dependent and IFN gamma associated. Weeks later, final elimination of HBV-infected liver cells occurs, presumably by a strong T-cell response with associated liver disease during acute self-limited HBV infection. The absence of a strong T-cell response is detected in patients with chronic hepatitis B. The precise mechanism that contributes to the failure of virus-specific T cell response is still not clear and is discussed by the authors.

Hepatitis B virus is the prototype virus of the family of *hepadnaviridae* that is further divided into the *orthohepadnaviruses* of the mammals and the *avihepadnaviruses* of the birds. The review of Funk *et al*[8], describes avian hepatitis B viruses as an important animal model for the understanding of the life cycle of *hepadnaviridae*. Since the discovery of duck hepatitis B viruses (DHBV) in 1980 many other *avihepadnaviruses* have been characterized in various bird species including cranes and herons. The genomic and structural organization is very similar in human HBV and DHBV. Like HBV, DHBV replicate their DNA genome by reverse transcription of an RNA intermediate. Similar to HBV is their narrow host range, e.g. DHBV infects only distinct duck and goose species but not chicken. Both viruses infect mainly hepatocytes and share a similar life cycle. Nevertheless there are differences that have to be taken into consideration when comparing results obtained from HBV or DHBV systems. First of all, the transmission of infection in ducks occurs mainly vertically, while horizontal transmission is uncommon in contrast to human HBV. Although nearly all of DHBV infected ducks develop chronicity, none or very mild hepatitis is detected and no hepatocellular carcinoma occurs. Furthermore, the DHBV envelope is composed of only two surface proteins [large (L) and small (S) surface proteins] and lacks the middle surface protein (MHBs) found in HBV and other *orthohepadnaviruses*. Unlike the envelope proteins of HBV, DHBV L and S-proteins are not glycosylated, but the DHBV L-protein is phosphorylated, a modification that is absent in HBV L-protein. Another difference is the open reading frame (ORF) for the X-protein. For DHBV, only a cryptic X-like ORF has been described, but apparently lacks a functional role in DHBV *in vivo*. Despite these limitations, DHBV infection of ducks has been an important animal model for the understanding of the biology of hepadnaviral infections, due to the ready availability of susceptible ducks.

Because of the various limitations of the duck hepatitis B infection model like absence of a strong immune pathogenesis leading to the development of liver cirrhosis and HCC, infection of woodchucks (*Marmota monax*) with woodchuck hepatitis B virus (WHV) represents a well-accepted model for many aspects of pathogenesis of human HBV infection. In their review, the authors Stephan Menne and Paul J. Cote describe in detail the woodchuck animal model of orthohepadnaviral infections[9]. WHV, as HBV, is a member of the genus *orthohepadnavirus*. In nature, transmission of the virus occurs vertically and horizontally. Similar to HBV, infections of adult woodchucks with WHV results mainly in resolution and only 5% of infected animals progress to chronicity. Experimental infection of newborn woodchucks results in up to 75% to chronic infection, which is similar to the high properties of neonates born to HBV-infected mothers to develop persistent HBV infection. Chronic WHV infection is associated with life-long active viral replication and disease progression to chronic hepatitis and HCC in these animals. However, unlike chronic HBV infections, there is no naturally occurring e-antigen to anti-e seroconversion and step-down of viral replication. The high viral replication and the very high load of surface antigen and continuous presence of e-antigen in chronic WHV-infected woodchucks might therefore play a major role in disease progression that leads to a nearly 100% risk of these animals to acquire a HCC. However, even after recovery from acute WHV-infection a higher risk of HCC remains (up to 20%), when compared to uninfected woodchucks. Interestingly, the authors could show that WHV replication could be reactivated in serologically long-term resolved adult woodchucks by immunosuppression with cyclosporine A. These experiments support the hypothesis that replication-competent WHV (or HBV in humans) is able to persist years after recovery from acute hepatitis and is controlled by an intact host immune system. The observation in the woodchuck model is supported by recently described fatal reactivation of recovered HBV-infected persons during active immune suppression. The woodchuck could therefore be a very helpful model to study the mechanism of a possible long life persistence of HBV even after serological recovery from acute hepatitis. However, compared to humans, the immune system of woodchucks is not well characterized. Nevertheless, the woodchuck model has very much expanded our knowledge of immune pathogenesis of acute and chronic hepadnaviral infections. Chronically WHV-infected woodchucks have proven to be an invaluable tool for preclinical screening of antiviral drugs against chronic HBV infections of men. Of the many studies with nucleoside and nucleotide analogues tested so far in the woodchuck, telbivudine and clevudine are the most potent with a 8 log10/mL reduction in serum viremia after daily administration of 10 mg/kg for 4 wk, respectively. In addition, continuous treatment with clevudine also delayed the development of HCC significantly. The antiviral activity of tenofovir is much lower under same conditions with only 1.2 log10/mL reduction in serum viremia after 4 wk and is more or less comparable to those of lamivudine and adefovir in the woodchuck model. Furthermore, the authors describe in detail further immunological studies in neonatal

and adult WHV-infected woodchucks that might help to identify the factors responsible for the switch from acute to chronic infection. First results showed that under some conditions, a combination of long-term antiviral drug treatment and therapeutic vaccination can break humoral and cellular immune tolerance in chronic WHV-infected woodchucks, when compared with antiviral monotherapy. The woodchuck animal model of chronic HBV infection is therefore an invaluable tool to study viral pathogenesis and host immune responses.

Despite the existence of a HBV vaccine, more than 370 million people are chronically infected with HBV worldwide, leading to chronic liver disease and development of HCC in many cases. Therefore the overall goal of an antiviral therapy for these cases would be the cure of chronic HBV. However with the current antiviral drugs this might not be achievable in all cases within the near future. In the review by Hans L. Tillmann, the aims of today's antiviral therapy against chronic HBV are summarized[10]. That is, first of all, prevention of liver disease and development of HCC in these patients by control and suppression of HBV replication. The author discusses in detail the different types of chronic HBV and their treatment parameters. For antiviral therapy, only interferon alpha (IFN α) and inhibitors against the reverse transcriptase of HBV are currently available. With standard IFN α, seroconversion from HBe to anti-HBe is induced in 20%-40% of patients 24 wk post-treatment. In chronic HBV patients with a HBeAg negative phenotype, patients showed a good response under IFN α treatment for 6-12 mo but a sustained response was usually not observed in the majority of cases. Unfortunately, longer treatment is usually limited by strong side effects, e.g. flu-like symptoms. Pegylation of IFN α, leading to a longer half life of the modified interferon has similar efficacy, but in general the sustained response for both, standard and pegylated interferon seems to be dependent on the HBV genotype. In contrast to the side effects of interferons, inhibitors of HBV reverse transcriptase can be used for prolonged antiviral therapy. Many of the nucleotides or nucleosides analogues against HBV were developed and used in HIV therapy. Lamivudine, also called 3TC, inhibits HBV reverse transcriptase very efficiently and usually results in viral suppression of 5-6 log10 copies/mL after one year of treatment. Prolonged Lamivudine treatment may contribute to seroconversion of HBe to anti-HBe within 50% of patients after 4 years of therapy. The major problem with prolonged Lamivudine, but also other reverse transcriptase inhibitors is occurrence of drug resistance due to the selection of HBV mutants. The mutations occur mainly within the YMDD-motive of the C-domain of the viral polymerase, however other mutations are also described. The high variability of the HBV reverse transcriptase together with a high replication rate of HBV and the slow kinetics of viral clearance lead to rapid selection of mutants under drug selection pressure that results in development of resistance during continuous therapy. For Lamivudine, up to 70% resistance has been described after 4 years of treatment. This leads to viral breakthrough and progression of liver disease. The author notes that it is therefore necessary to perform early diagnostics of drug resistance and to adapt antiviral therapy prior to breakdown of liver function. Even more important is that the new antiviral in use must not show cross-resistance to the HBV mutants selected by the former antiviral, e.g. telbivudine and emtricitabine have been reported to show a similar resistance mutation profile like Lamivudine.

For the future, it will be necessary to have antivirals on hand that inhibit different steps of the viral life cycle. Rational targets might be inhibitors of attachment and entry, conversion from rcDNA to cccDNA, capsid assembly, envelopment and secretion of viral particles. For attachment and entry, it has been shown that acylated preS1 peptides block HBV infection *in vitro* and in animal models. Based on these results a potent inhibitor of viral infection (Myrcludex B) is currently being developed.

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