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The Chimpanzee Model

Contributions and Considerations for Studies of Hepatitis B Virus

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1. Introduction

Efforts to control the global pandemic of human hepatitis B virus (hHBV) infection have been hampered by incomplete understanding of viral–host interactions in this disease. This situation has been confounded by the fact that hHBV has a limited host range and cannot be propagated in simple cell culture ***(1)***. Reproducible experimental infection with determination of infectivity was demonstrated in chimpanzees (*Pan troglodytes*), but not other primates ***(2–4)***, long before other animal models such as the woodchuck were identified. After successful inoculation of chimpanzees was reported in 1972, multiple institutions, including a multigroup collaboration between the FDA, CDC, and NIH, initiated studies to evaluate them as a model for the study of HBV. For the majority of studies only chimpanzees "with no prior exposure" to virus were used because those with positive serology [either from exposure to hHBV or chimpanzee HBV (chHBV)], with an estimated prevalence of 3–6% in Africa ***(5)***, were not reproducibly susceptible to infection ***(3)***. It has been widely reported that the effects of HBV infection in chimpanzees are milder than in humans, that is, few have developed fulminant hepatitis, and inoculated chimpanzees exhibit few symptoms or signs of infection. Furthermore, the incidence of chronic infection with HBV (*see* Table 2) and horizontal and vertical transmission (from mother to offspring) in chimpanzees is lower than in humans ***(6,7)***. Chimpanzees have been the cornerstone of all research on infectivity of HBV and safety and efficacy of vaccines.

Chronic HBV infection occurs in approx 5% of experimentally infected chimpanzees, defined as persistence of hepatitis B surface antigen (HBsAg) for $>$ 12 months (approx ⅔ clear HBsAg between 6 and 12 months of infection) ***(8)***. Liver biopsies of chimpanzees with chronic HBsAg have never been reported to have more severe abnormalities than mild persistent hepatitis ***(8–11)***, but we were unable to find published

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reports of liver biopsies performed later than 36 mo after inoculation with HBV, and found few reports of chimpanzees with chronic infections of unknown source ***(12–14)***. Likewise, liver biopsies performed since 1972 have not been reexamined for the presence of covalently closed circular DNA (cccDNA) or integrated HBV-DNA, indicative of the chronic carrier state, in hepatocytes. In addition, other tissues that could harbor HBV ***(15)***, such as lymphocytes ***(9–11)*** and splenic tissues ***(16)***, are generally not available from these animals. Thus, it has not been possible to make histological and molecular comparisons between humans and chimpanzees with either serologically resolved or chronic infections. This information would be particularly interesting because of the demonstration by Penna ***(17)*** of the persistence of cccDNA in hepatocytes of humans with apparently "resolved" infections, which indicates that HBV can persist at low levels chronically, even in the absence of traditional serological markers. These issues raise questions about the utility of the chimpanzee model to predict the outcome of chronic infection, including cirrhosis and hepatocellular carcinoma, and the long-term efficacy of currently available treatments and vaccines. In addition, the only reported cases of hepatocellular carcinoma in chimpanzees involve two animals that had been experimentally infected with hepatitis C virus ***(18)***.

This chapter recounts the history of HBV studies in chimpanzees and explains the importance of the chimpanzee model to the study of human HBV disease and to HBV vaccine development, comparing and contrasting both host and viral factors, and revisiting the potential issues about the utility of this model for future research in the area.

2. Appreciation of Multiplicity of Infectious Agents Capable of Causing Hepatitis in Chimpanzees

Hepatitis of apparent viral origin in nonhuman primates was first described many years before HBV was identified in 1966 ***(19)***. There are reports in the 1930s and 1940s of jaundice following parenteral administration of blood products ***(19)*** or injection of a yellow-fever vaccination prepared in Rhesus monkeys ***(20)***. Between 1958 and 1960 there were multiple reports of primate-related spontaneous hepatitis (approx 26 clusters involving at least 106 veterinary personnel), associated primarily with exposure to chimpanzees rather than other primates ***(20)***, with as many as 43% in some groups infected (e.g., workers at Holloman Air Force Base ***[21]***). It was rapidly recognized that infected workers had handled chimpanzees newly arrived from Africa, indicating that the chimpanzees had only recently contracted the illness, as none of the chimpanzees quarantined for longer than 3 mo showed evidence of hepatitis. Microbiological analysis of stool, urine, and sera demonstrated that many of the infected chimpanzees had become ill with viruses such as reo-1, polio-1, ECHO-8, adenovirus 14 and 17, and an "unknown" type. This raised the question of whether chimpanzees, known to have occasionally been inoculated with pooled human sera after capture by animal dealers to protect them from development of human infections ***(21)***, had, in fact, developed significant infectious sequelae to this practice. The "unknown" infectious agent(s) remained elusive, but it was clear that there was both intercage transmission and transmission to veterinary personnel, indicating that fecal–oral transmission of what was later identified as hepatitis A virus (HAV) was most common in this period.

One chimpanzee at the Delta Regional Primate Research Center developed fulminant hepatitis 1 mo after arrival at the facility and two workers developed significant hepatitis, 4 and 7 wk after that chimpanzee became ill ***(20)***. Both humans and the chimpanzee had "unusual" liver histology, consistent with "acute yellow atrophy." Although this may have been an infection with HBV, this was not confirmed. In another early study ***(22)***, three of six chimpanzees inoculated with human serum from inhabitants of the Willowbrook State School (New York) developed evidence of hepatitis; although this serum may have contained more than one hepatotropic virus, HBV was later identified to be endemic in this population. The first case of documented transmission of HBV from chimpanzee to human was reported in 1970 after a cross-circulation experiment between a young girl and a chimpanzee positive for HBsAg ***(23)***. The specific medical history of the infected chimpanzee was not included, so the source of HBV is unknown. In 1972, Maynard demonstrated that serologically negative chimpanzees could be infected with serum derived from humans infected with HBV ***(3)***. The issue of whether apes could represent a reservoir for the human infection was briefly addressed but the prevailing attitude was that all chimpanzees serologically positive for HBV had been infected by human viruses prior to their arrival at research facilities.

One of the confounding factors to discriminate between incidence and prevalence of HBV and other forms of hepatitis in the natural habitat is that the vast majority of captive chimpanzees arrived in the United States with poor records, if any at all. Similarly, the precise geographic origin of the animals was unknown. In addition, all animals were treated as belonging to the same population. Based on recent population genetic surveys, it is now clear that some chimpanzee populations have been separated for long evolutionary periods ***(24)***, and that when using these animals for biomedical studies, it may be relevant to identify the geographical origin of the animal ***(25, 26)***.

The assignment of chimpanzees currently in NIH facilities (230 founder animals) into one of the four possible subspecies based on sequencing of mitochondrial DNA is still incomplete (Ely and Gagneux, *unpublished observations*). Preliminary results suggest that the vast majority originate from western Africa (West of the Niger River), but a smaller number of animals were also imported from central and East Africa, e.g., imported from Cameroon ***(21)*** and Rwanda ***(27)***.

3. Use of Chimpanzees for HBV Research

Early studies of hepatitis involved large groups of humans (U.S. armed forces recruits) and many individuals who would today not be considered suitable candidates for human research because of ethical considerations, such as children at institutions for the mentally ill (e.g., the Willowbrook State School) ***(28)***. Subsequently efforts to develop animal models were undertaken.

Although there were multiple reports of chimpanzees positive for HBV on arrival to captive facilities (**Table 1**), it was soon appreciated that serologically negative animals were susceptible to HBV infection. Maynard reported that as many as 55% of captured chimpanzees had HBV antibodies ***(3)***, and two animals that did not were susceptible to infection with a 1:10 dilution of human serum containing HBsAg. Several groups expanded these observations to demonstrate that most serologically negative chimpanzees are susceptible to experimental inoculation with HBV ***(3,4,29)***.

Table 1
HBV Prevalence Studies of HBV in Newly Captured Chimpanzees Housed in U.S. Facilities

	Positive/surveyed HBsAg	HBV antibodies	Site
1969 ***(117)***		3/62	Holloman AF base, LEMSIP
1971 ***(118)***	6/97	29/97	Phoenix Labs
1972 ***(119)***		46/81	Holloman AF Base
1980 ***(120)***	2/82	24/82	Southwest Foundation

All studies used serological methods for the detection of antibodies, but not all assayed for the presence of viral antigen. No retrospective analysis of these specimens has been performed to discriminate whether the infectious agent was chHBV or hHBV.

Chimpanzees selected for experimental studies were all negative by serologic assay to HBV and had no known exposure to blood, blood products, or plasma derivatives. In many cases liver enzyme assays and liver biopsies were performed to qualify the animals as noninfected. Radioimmunoassays were widely used after the mid-1970s and significantly improved detection levels. However, it is now known that none of these selection criteria rule out previous infection because infectious episomes can be detected in hepatocytes in humans serologically negative for HBV ***(17,30,31)***.

Apart from polio vaccine safety and other studies done on 300–400 chimpanzees at Lindi Camp near Stanleyville in the Belgian Congo in the 1950s, which included preliminary studies by Deinhardt and colleagues on hepatitis, HBV research was the first large-scale application of the chimpanzee in biomedical research ***(32)***.

The reaction of chimpanzees to inoculation with human serum positive for HBsAg varied between animals, but appeared to be consistently milder than in humans ***(4,8,29)***. Despite the inoculation of hundreds of animals, the first cases of confirmed fulminant HBV infection were reported in 1993 ***(33)***. Antibody production, changes in liver enzyme values, and changes in hepatic tissue architecture have been clearly documented and appear to follow a time course similar to that in humans. Transmission studies, reinfection, and combined infection (superinfection) with more than one hepatotropic virus have been carried out. In addition to their importance to the study of infectivity of HBV and in HBV vaccine safety and efficacy trials, the use of chimpanzees was instrumental in the identification of hepatitis C virus (HCV) ***(34)***.

4. Safety Testing (the "Chimpanzee Assay")

The reproducibility of infection and its temporal sequence in chimpanzees made it possible to use chimpanzees as an "assay" for the presence of HBV in human serum and serum-derived products, such as immunoglobulins and clotting factors. Between 1980 and 1993 multiple methods for decreasing infectivity of serum were reported, each of which used chimpanzees in traditional experimental format, with some animals serving as control (untreated product) and others receiving the treated product or, alternately, treated animals serving as their own controls. These studies were pivotal for reducing

viral contamination of blood and serum products, and for determining the activity of serum proteins after various treatments. Specific agents tested included antibodies to HBV ***(35,36)***, ultraviolet irradiation ***(37)***, urea/formalin treatment of human sera ***(38)***, Tween-80 treatment ***(39)***, a combination of Tween-80–propiolactone and UV irradiation ***(40,41)***, chloroform ***(42)***, Tween-80–20% ether and cold (4°C) treatment ***(43)***, glutaraldehyde ***(44)***, heat treatment ***(45,46,47)***, ion exchange treatment ***(48)***, photochemical treatment ***(49)***, and disinfectants (two quaternary and one phenolic) ***(50)***. Later, when the polymerase chain reaction (PCR) became widely used, it became the preferred method for detection of HBV contamination.

Chimpanzees were also used to assay the presence of trace amounts of HBV in vaccine lots. Chimpanzees born in captivity to mothers serologically negative for HBV were the principal animals used until the U.S. moratorium on breeding in captivity came into effect in 1998. Despite improvements in the sensitivity of assays for markers of HBV, it is now recognized that an indeterminate number of animals must have escaped detection as a result of false negative assay results.

5. Experimental Inoculation of Chimpanzees with HBV

The earliest studies (**Table 2**) demonstrated that infectivity was related to both dose and serotype of HBV; inocula were diluted in fetal calf sera serologically negative for HBV. Later reports described neither the serotype nor precise source of HBV, so it is possible that the inocula for many of the experiments could have contained more than one hepatotropic virus. Infectivity of HBV inocula was calculated by the Reed–Muench method ***(8)*** and a high percentage of infected animals were achieved using an inoculum of 10^7–10^8 CID_{50} (chimpanzee infectious doses)/mL for subtypes adw, ayw, adr, and 10^0–10^3 CID_{50} for 1 yr ***(4)***. Interestingly, no such differences in infectivity were apparent in the human host. There was a roughly inverse relationship between the amount of virus inoculated and the time to appearance of HBsAg in chimpanzees, with some serotypes exhibiting more reproducible incubation times than others. The longest incubation time was 19 wk, which was comparable to infections in humans.

Animals successfully infected have typical biochemical, serological, and histological patterns of mild type B hepatitis and responses are not distinguishable based on viral subtype. Barker ***(29)*** reported that 27/29 chimpanzees developed HBsAg, which persisted in 2/29; antibodies to HBsAg were detected in 24/29 and antibodies to HBcAg in 23/29. As can be seen, and calculated from **Table 2**, the rate of infection of susceptible chimpanzees is approx 80–90%, with variation probably based on viral titer of inocula, as early studies reported complete susceptibility to infection of seronegative chimpanzees ***(3,51)***. This rate of infectivity is similar to that reported in human populations with endemic infections, such as the Willowbrook School population, where 90% of children housed at the center for 3–5 yr had detectable antibodies to HBV ***(52)***. Karasawa et al. ***(9–11)*** and others have carefully documented histologic features of mild hepatitis. Notably, there have been no reports of hepatocellular carcinoma in HBV-inoculated chimpanzees. Of the more than 150 chimpanzees reportedly infected with HBV from various sources **(Table 2)**, detection of chronic HBsAg in serum, using standard serologic assays, has occurred in $< 5\%$ of cases, or 5 of >150 reported [one addi-

Table 2
Infectivity Studies of Chimpanzees Previously Unexposed to HBV or to Human Serum Products

Date	No.	HBV type	Route	HBsAg	HBsAb	Histologic changes of hepatitis	Persistence of HBsAg	F/U
1962 ***(32)***	6	WB serum (?MS-2 strain)	IP	NR	NR	3/6	NR	2 mo
1972 ***(3)***	2	hHBV-plasma	IV	1/2	2/2	0/2	0/2	>1 yr
1973 ***(4)***	6	hHBV-plasma (NIH) and partially purified plasma (SQ)	SQ	5/6	5/6	2/5	0/6	20–44 wk
1973 ***(5)***	3	Serum from hemophiliac with chronic HBV	IV	1/3	1/3	NR	NR	6 mo
1974 ***(121)***	8	hHBV (MS-2) and chimp serum from infected chimps	IV	8/8	6/8	NR	1/8	2 yr
1974 ***(122)***	4	HBV	IV	3/4	3/4	3/4	0/4	6 mo
1975 ***(123)***	6	HBV + cytoxan	IV	6/6 (one spontaneous infection)	4/6	6/6	2/6 treated with cystoxan during primary infection were positive at death at 11 and 42wk	13 mo
1975 ***(51)***	12	NIH plasma pool, MS-2 strain	IV	9/12	12/12	8/12	2/12	NR
1975 ***(124)***	2	MS-2 strain	IV	2/2	2/2	2/2	0/2	9 mo
1975 ***(29)***	34	hHBV: 4 serotypes; ayw strain was MS-2	IV	27/34	29/34	23/29	1/34	7–16 mo

1977 ***(125)***	4	HBV-saliva, semen	IV	2/4	2/4	2/4	NR	6 mo
1977 ***(126)***	7	Serum with HBeAg or anti-HBeAg	IV	4/4	1/3	NR	NR	NR
1977 ***(127)***	1	hHBV+ SQ and IV ethanol	IV	1/1	1/1	0/1	0/1	6 mo
1979 ***(128)***	3	hHBV	IV	3/3	3/3	NR	0/3	22 mo
1979 ***(129)***	1	HBV human plasma	IV	1/1	1/1	0/1	0/1	1yr
1979 ***(130)***	1	hHBV (pooled serum)	IV	1/1	NR	NR	—	9 mo
1980 ***(131)***	9	hHBV, multiple sources	IV	8/9	9/9	NR	1/9	>2 yr
1980 ***(76)***	7	JHB 001 hHBV	IV	7/7	7/7	7/7	1/7	3 yr
1982 ***(132)***	1	huHBV (plasma)	corneal	1/1	NR	NR	NR	9 wks
1982 ***(133)***	1	HBV2,6,14; cloned	IV,IM,IH	1/1	1/1	0/1	0/1	1 yr
1985 ***(134)***	8	Varied sequences, routes of inoculation	IV,IP	3/6	3/6	3/6	ND	1 yr
1985 ***(8)***	6	JHB001	IV	6/6	6/6	5/6	0/6	>2 yr
1986 ***(135)***	3	hHBV variants (hu sera + anti-HBc, anti-HBe, -HBsAg)	IV	4/4	4/4	4/4	0/4	1 yr
1987 ***(136)***	2	Media from HEPG2 cells transfected with HBV	IV	2/2	1/2	2/2	1/2	NR
1988 ***(137)***	1	Media from HEPG2 cells transfected with HBV	IV	1/1	NR	1/1	NR	NR
1988 ***(138***	2	Plasma from HBsAb-negative patients	IV	2/2	2/2	1/2 (2/2 by PCR)	0/2	8 mo
1990 ***(139)***	1	Media from rat hepatoma cell line transfected with HBV	IV	1/1	1/1	NR	0/1	8 mo

Table 2
Infectivity Studies of Chimpanzees Previously Unexposed to HBV or to Human Serum Products

Date	No.	HBV type	Route	HBsAg	HBsAb	Histologic changes of hepatitis	Persistence of HBsAg	F/U
1990 ***(30)***	1	hHBV from serologically negative, PCR + individual	IV	1/1	1/1	0/1	PCR +	17 mo
1993 ***(33)***	3	hHBV mutated in pre-core region	IV	3/3	3/3	NR	0/3	1yr
1997 ***(140)***	6	hHBV, Arg-Gly at codon 145 S	IV	5/6	5/6	ND	0/6	24 wk
2001 ***(73)***	3	Serum/lymphocytes from patients HBV DNA + by PCR, HBsAg-	IV	0/3 (by PCR)	ND	ND	ND	55 wk
Total	154							

As explained in the text, the preinoculation history of most captive chimpanzees was limited. The rate of infectivity was >80%, as determined by detection of viral antigen and antiviral antibodies. Many studies did not examine liver histology, but those that did reported changes consistent with mild hepatitis. A calculation of percentage of infected animals that develop chronic infections is difficult because duration of follow-up was extremely limited, in most cases less than the 12 mo required for viral clearance in chimpanzees. NR indicates not reported.

tional case was detected using PCR in a serologically negative animal ***(30)***]. This percentage of chronically infected chimpanzees remains constant even if the calculation is restricted to animals followed for longer than 1 yr, which is the minimum follow-up required to ensure that spontaneous clearance of HBsAg has not occurred ***(8)***.

Infection with more than one hepatotropic virus has been reported to be associated with altered response to infection with HBV ***(53,54)***. Brotman et al. ***(55)*** reported that chimpanzees inoculated with "standard" doses of HBV have 100% antigenemia with HBsAg (18/18), with 15/18 having at least one abnormal alanine aminotransferase (ALT). However, simultaneous exposure to non-A, non-B, and HBV in seven animals yielded milder results: five of seven developed HBsAg, in each case with a greatly delayed onset, and three of seven had only borderline ALT abnormalities. Kos et al. ***(56)*** demonstrated decreased levels of HBV in a chronically HBV-infected chimpanzee inoculated with HDV. However, Dienes et al. ***(14)*** reported that chronic HBV carriers experimentally infected with either HAV or non-A, non-B agents developed more severe disease than infected native animals. This indicates that the number of animals that have been experimentally (knowingly) infected with more than one hepatotropic virus is too low to allow definite conclusions.

6. Reinfection of Chimpanzees with HBV

The presence of detectable antibodies to HBV was found to be associated with resistance to experimental reinfection with HBV. In 1974, Wilson and Logan ***(57)*** reported two chimpanzees with low antibody titers to HBV who did not develop detectable circulating HBsAg after injection of highly infectious serum; in fact, antibody titers increased substantially. In 1975, Maynard et al. ***(51)*** reported that chimpanzees reinfected with HBV of a different serotype did not develop hepatitis. Furthermore, Trepo et al. ***(58)*** demonstrated the presence of Arthus reactions in 7/7 animals with measurable anti-HBV that had been immunized 1 yr earlier. These observations formed the basis for vaccine development. Early studies demonstrated that immune function was directly related to outcome of infection. Wilson reported a chimpanzee treated with cyclophosphamide and prednisone around the time of challenge with HBV serum, and with prednisone for 7 wk when HBsAg levels decreased. At necropsy, abundant Dane particles were present in both serum and liver, implying that effective immune response to infection had been blunted by treatment with prednisone. In contrast, reinfection with HDV, even if associated with antibodies to HDV, has been reported ***(59)***.

7. Sequelae of Infection with HBV in Chimpanzees

Studies to determine hepatic sequelae of chronic infection with HBV in chimpanzees have been sparse because of the generally short duration of follow-up in most experimental infection studies. However, it was appreciated as early as 1982 ***(60)*** that in chronically infected animals, HBV-DNA existed in a covalently closed, supercoiled circular configuration (cccDNA) not integrated into the host genome, and was infectious. Thus, although no findings beyond minimal hepatitis have been recorded in the 1- to 3-yr follow-up of the inoculation experiments, the follow-up may have been insufficient to document chronic changes. There are several reports of biopsies performed in chron-

ically infected chimpanzees. Krawczynski et al. ***(12)*** described liver biopsies in four chimpanzees between the ages of 6 and 15 yr who were positive for HBsAg, due to unknown exposures, for 2–8 yr; all had histopathologic features consistent with "minimal hepatitis" of humans. Similarly, Shouval et al. ***(13)*** reported liver biopsy results from five chimpanzees with chronic HBsAg (the source of the infectious inoculum was known in only two animals, infected for a minimum of 5 and 10 yr); only one of the specimens showed borderline findings for chronic aggressive, or active, hepatitis. Dienes et al. ***(14)*** described liver biopsies from seven carrier chimpanzees, with mild activation of sinusoidal cells, rare and mild fibrosis of portal tracts, and slight proliferation of bile ductules. If the pathology from these 12 chronically infected chimpanzees is representative of the course of infection in the general population, it appears unlikely that chimpanzees chronically infected with HBV would develop cirrhosis or hepatomas, as occurs with greater incidence in chronically infected humans and in chimpanzees infected with HCV ***(18)*** or chronic *Schistosoma mansoni* ***(61)***.

8. Vaccine Trials in Chimpanzees

The discovery that animals previously exposed to HBV were not susceptible to reinfection opened the way to the study of the immune modulation of infection. The first vaccines (**Table 3**) involved injection of the empty 22-nm particles purified from the plasma of chronically infected carriers. To minimize the risks of infection during immunization, multiple steps were taken to purify human plasma containing HBV, including centrifugation, fractional precipitation, chromatography, and molecular exclusion. To inactivate the HBV, various detergents, formaldehyde, heat, pH changes, and ultraviolet radiation were used. Within a few years after successful inoculation of a chimpanzee with HBV, there were multiple vaccines based on noninfectious 22-nm subviral particles (containing mostly S antigen) isolated from chronic human HBV carriers. The initial findings were somewhat confusing, perhaps because different evaluation criteria were applied to the studies, but sufficiently promising that second generation vaccines were developed. Unfortunately, all published studies used unique injection schedules regarding number of boosters and timing of injections.

The second generation of vaccines contained recombinant subviral particles produced in stably transfected eukaryotic cell lines. The sources of subviral particles for vaccine development included plasma-derived polypeptides and synthetic polypeptides expressed in *E. coli,* yeast, and murine cells. Live recombinant adenovirus vaccines were introduced in 1989 ***(62)*** but both these and synthetic polypeptides did not prove to be effective. It was recognized in the late 1980s that the M and L proteins, containing the pre-S region of HBV, in addition to the S protein, were essential for vaccine effectiveness ***(63)***. The latest generation of vaccines and treatments are intended to target the HBV carrier state.

Chimpanzees have been used successfully for all vaccine safety and efficacy trials ***(64–66)***. Cross-protection afforded by antibodies induced by different HBsAg subtypes ***(51,67)*** was also first demonstrated in chimpanzees. Antibodies to the viral envelope confer protective immunity; and 10 mIU/mL is considered sufficient to confer immunity. HBcAg is highly immunogenic but although anti-HBcAg protects chimpanzees

Table 3
HBV Immunization Studies Conducted in Chimpanzees

Date and first author	Type of vaccine	Number of chimpanzes	Failure rate	Duration of follow-up
1971 ***(118)***	Hypersensitivity test purified HBsAg	2	—	—
1974 ***(120)***	Partially purified chimpanzee HBsAg	2	—	
1974 ***(141)***	Vaccine safety HBsAg +/– CFA	28	—	—
1975 ***(58)***	Vaccine safety HBsAg purified + CFA	15		1 mo
1975 ***(64)***	HBsAg 22-nm CsCl gradient purified, formalin inactivated	7	1/4	6 mo
1976 ***(142)***	Ad and ay HBsAg from pooled serum	10	1/16	10 mo
1978 ***(143)***	HBV polypeptide vaccine efficacy	4	—	3 mo
1978 ***(144)***	Bivalent NIH vaccine HBeAg active and passive immunization	7	—	—
1978 ***(145)***	HbsAg vaccine efficacy	2		
1978 ***(146)***	Safety and efficacy NIAID subunit vaccine	16	0/4	6 mo
1981 ***(147)***	Vaccine efficacy bivalent ad/ay HBsAg (Hepagen)	46	4/46	32 mo
1982 ***(148)***	Vaccine efficacy HBeAg and HBsAg	4	—	
1983 ***(149)***	Vaccine efficacy for transfusion protection HBsAg from pooled serum	8	3/4	16 mo

Continued

Table 3
HBV Immunization Studies Conducted in Chimpanzees

Date and first author	Type of vaccine	Number of chimpanzes	Failure rate	Duration of follow-up
1983 ***(150)***	Heptavax B safety	—		6 mo
1984 ***(151)***	Vaccine efficacy HBsAg ayw MS-2 10^8 PFU (Hevac B, Pasteur)	42	—	6 mo
1984 ***(152)***	Vaccine efficacy recombinant vaccinia virus intradermal	2	0/2	8 mo
1984 ***(153)***	Treatment safety IgG anti-HBcAg and anti-HBcAg	10	—	12 mo
1985 ***(68)***	Vaccine efficacy recombinant HBcAg in *E. coli* and adjuvant	5	1/3	16 mo
1987 ***(47)***	Inactivation safety human HBsAg-plasma	4	0/4	5 mo
1987 ***(154)***	Vaccine efficacy recombinant HBsAg in yeast	7	0/4	—
1988 ***(155)***	Vaccine efficacy cloned adw 226 aa HBsAg adw & alum adjuvant (Amgen)	6	0/5	12 mo
1989 ***(62)***	Vaccine efficacy oral live recombinant adenovirus	3	1/2	11 mo
1990 ***(63)***	Vaccine efficacy recombinant pre-S and S HBsAg adr in yeasts	9	0/8	10 mo
1993 ***(50)***	Test of disinfectants	4		7 mo

1997 ***(156)***	Vaccine efficacy for offspring of chronic carriers DNA vaccine pCMV S2-S	3		16 mo
1997 ***(138)***	Vaccine efficacy recombinant HBsAg effective against surface mutant	6	0/4	8 mo
1998 ***(157)***	Vaccine efficacy Pre s1 pre-S2 and S in mouse c1271 cells	5	0/3	9 mo
1998 ***(71)***	DNA vaccine safety and efficacy recombinant retrovirus (Chiron)	3 chronic carriers of chHBV	HBV DNA down	12 mo
2001 ***(70)***	Treatment efficacy/safety chronic carrier DNA prime/canary pox boost	1 chronic carrier		20 mo
	Total	>250		

Most studies did not include information about the animals used in the study, such as the previous experimental studies for which the chimpanzees had been used. The failure rate was essentially negative after 1985. The few studies that have tested vaccines in chronic chimpanzee carriers of HBV are noted. The duration of follow up, when included, after vaccine injection was universally short. (—) indicates information not included in publication.

from challenge from live HBV, high titers of maternal antibodies to HBc fail to protect the few infants of chronically infected mothers from perinatal infection ***(68)***.

In the past 25 yr, more than 250 chimpanzees have been used in vaccine safety and efficacy trials. The record in terms of safety is impressive: not a single batch of tested vaccine appeared to have contained infectious HBV. In terms of efficacy, there was a rather steep learning curve, with high efficacy for all trials since the mid-1980s. However, the number of animals involved in individual vaccine efficacy trials has been very limited, owing to the difficulty and high cost of keeping large numbers of chimpanzees. Some of the earlier trials that had used more than 40 animals reported failure rates of up to 25%. The current failure rate for HBV vaccine in humans is substantially lower (5–10%), which highlights the importance the chimpanzee has had in protecting humans at risk, or with chronic infection, with HBV.

The cost of producing the currently available vaccines has precluded generalized use in poor nations. There are recent promising reports for treatment of chronic HBV infection using DNA vaccines. This method is attractive because the response induces cytotoxic T lymphocyte (CTL) and antibody responses to the same level as the most successful subunit vaccines. All current vaccine trials have used small numbers of chimpanzees because it is so rare to have chronic chimpanzee carriers: most infected after birth spontaneously resolve infection with HBV ***(6)***. Davis et al. ***(69)*** used pre-S2 + S of ayw strain, with boost of S, adw subtype at 52 wk and demonstrated early high titers against pre-S2 domain (10-fold higher against M and S envelope proteins than those composed entirely of S), but did not subsequently challenge with HBV. The Pancholi et al. study ***(70)*** used a chimpanzee inoculated with HBV in 1985, with persistent positive serology for 12 yr, and injected first HBsAg-encoding plasmid, followed by boost with recombinant canarypox virus encoding HBsAg, preS-1, and pre-S2. There was a decline in HBV DNA coincident with increase in interferon-γ (IFN-γ)-secreting cells. The Sällberg et al. study ***(71)*** used three chimpanzees chronically infected with HBV, at least one of which almost certainly was infected with chHBV. The Sällberg group used a recombinant vector expressing HBC core antigen–neomycin phosphotransferase II fusion protein. Although two of the three animals showed no change in HBV viral load, the third animal developed antibodies to HBV core antigen and decreased HBV DNA levels. Further studies are required to develop a safe and effective vaccine with production costs that will be conducive to widespread use in the chronic carrier human population.

9. Potential Problems with the Chimpanzee Model

There are many unresolved issues raised by the review of the studies thus far performed using chimpanzees. The first is that the tests performed between 1969 and the mid-1980s to determine "susceptibility" of chimpanzees to infection with HBV were insensitive, and can safely be assumed to have produced many false-negative results, despite the fact that many included preinoculation serology, biopsy, and chemical analysis. Animals could have previously been exposed and yet been serologically negative, and there has been more than one report of transmission of HBV from serologically negative, but PCR positive, humans to chimpanzees ***(72)***. With the benefit of

hindsight it would not be unrealistic to posit that infectious particles could have been recovered from serologically negative chimpanzees, had an organized effort been undertaken, or if universal archiving of biopsy and serum specimens had been applied. Another (troubling) possibility is that the animals could have been chronically infected in the wild with chHBV variants carrying mutations in the S region, as epitopes in this area mediate recognition of HBsAg in both humans and chimpanzees ***(73)***. Such mutations have so far not been reported in chHBV, but we have only a minute sample of the existing chHBV diversity documented, as complete genomic sequences have been completed from only 11 chHBV sequences **(Table 4)**. Such mutations are likely to be one reason for the failure, albeit small, of currently used vaccines. The existence of similar antigenic mutants in chimpanzees, because they manifest few to no clinical signs of infection, could have gone unnoticed. If a large number of chimpanzees had been previously exposed to HBV, or if the inocula contained a mixture of hepatotropic viruses, experimental reinoculation would have altered the course of infection ***(14,55)***. Furthermore, rather than simply interfering, reinoculation could reactivate a latent infection as shown by Bock et al. ***(74)***. Therefore, conclusions made about the course of infection with HBV in chimpanzees have been compromised by insensitive serologic studies, lack of archived serologic and pathologic specimens for reanalysis, and, most of all, generally limited duration of experimental follow-up.

A second issue regarding the value of the chimpanzee model relates to the applicability of the model data to human infection with HBV. Although infectivity of HBV is similar between chimpanzees and humans, the course of the infection in chimpanzees is milder than in humans, and vertical transmission, the major transmission in humans, in chimpanzees is rare; in addition, horizontal transmission seems clearly limited in captive chimpanzee populations ***(6)***. The experimental conditions for inoculation of chimpanzees may not mirror those in which humans are infected, because humans may be infected repeatedly and with mixed hepatotropic viruses. When chimpanzees were simultaneously infected with HCV and HBV the result was a milder infection ***(55)***, but also metachronous infections resulted in more significant infections ***(14)***. This said, when infection with HBV occurs in a naive individual, both humans and chimpanzees exhibit a biphasic response ***(75)***, and both phases are milder in chimpanzees. As can be seen in **Table 2,** substantially fewer chimpanzees (<5% in published studies) with chronic infections have been reported than in humans. This indicates that there are fundamental differences in immune response between humans and chimpanzees with regard to HBV.

A third problem is that the total number of chimpanzees infected has been too small to address the major public health risk associated with HBV, which is the number of chronic human carriers of the virus (approx 350 million) in the world. Even if one were able to identify all chronic chimpanzee carriers, the total is almost certainly too small to use for efficacy testing of all candidate therapeutic vaccines. Chimpanzees chronically infected with chHBV would probably have comparable responses as carriers for hHBV, based on the reports of vaccine efficacy in a small population of chimpanzee carriers that had almost certainly been infected with chHBV. However, it is not clear that the group of chronic carrier chimpanzees can be identified.

Table 4
Chimpanzee Hepatitis B Viruses (chHBV) for Which Complete Genome Sequence Information Exists

GenBank accession number	Chimpanzee subspecies	Facility	Authors	Virus name/ Serotype	Host alive or dead
DOOO22O	*P.t. verus*	London Zoo	Vaudin et al. ***(82)***	LSH Chimp K	Dead?
AF222322	*P.t. troglodytes*	CDC	Hu et al. ***(77)***	HBV CH109	Dead?
AF222323	*P.t. verus*	CDC	Hu et al. ***(77)***	HBV CH926	Dead?
AB032431	*P.t. verus*	Vilab Liberia	Takahashi et al. ***(78)***	HBV/E-ch195	Dead?
AB032432	*P.t. verus*	Vilab Liberia	Takahashi et al. ***(78)***	ChHBV-Ch256?	Dead
AB032433	*P.t. verus*	Vilab Liberia	Takahashi et al. ***(78)***	ChHBV-Ch258	Dead?
AF242585	*P.t. troglodytes*	Cameroun	MacDonald et al. ***(79)***	HBV Chimp 2	Alive
AF242586	*P.t. verus*	Univ of Edinburgh	MacDonald et al. ***(79)***	HBV Chimp4	Alive
AB046525	*P.t. troglodytes*	Gabon	Takahashi et al. ***(78)***	PttHBV Ch Bassi	Alive?
AF305327	*P.t. vellerosus*	Coulston Foundation	Hu et al. ***(25)***	ChHBV CB0376	Alive
AF305326	*P.t. verus*	Coulston Foundation		CB0031	Alive
AF305328	*P.t. troglodytes*	Coulston Foundation		CH116	Alive
AF305329	*P.t. verus*	Coulston Foundation		CH1435	Alive
AF305330	*P.t. verus*	Coulston Foundation		CH1436	Alive
AF498266	*P.t. schweinfurthii*	wild	Vartianan et al. ***(80)***	Chimp FG	Dead

The number of chimpanzees currently housed in U.S. facilities chronically infected with chHBV is at least eight; the number of chimpanzees chronically infected with hHBV is more difficult to determine because of the short follow-up of infected animals noted in **Table 2**.

Finally, ethical considerations have led many scientists to reconsider the use of chimpanzees for invasive biomedical research. The European Union has passed laws seriously limiting biomedical research on chimpanzees and other primates. The public acceptance for large-scale biomedical studies on chimpanzees is likely to be low in North America and Japan. In the United States several large facilities with captive chimpanzees have closed, and in 2000 Congress passed a "Chimpanzee Health Improvement, Maintenance, and Protection (CHIMP) Act," which permits "noninvasive behavioral studies of the chimpanzees, or medical studies conducted during the course of normal veterinary care that is provided for the benefit of the chimpanzees" ***(76)***. The use of the conjunction "or" indicates an acceptance that some studies that are not beneficial may be performed on chimpanzees.

10. Relevance of Distinct Chimpanzee HBV (chHBV) to the Chimpanzee as a Model for Human HBV Infection

Many investigators have explored the differences in genetic sequences of HBV to understand individual differences in viral handling. Since it had been recognized in the early 1970s that previous infections with HBV were at least potentially protective against reinfection, the presence of an endemic infection in the study population, in this case chimpanzees with chHBV, would be highly relevant to their use as a model for human disease. The discovery of chimpanzee HBV in 2000 gave a partial explanation for the large number of chimpanzees found to be serologically positive for HBV upon arrival to captivity. Two retrospective studies used banked sera from chimpanzees positive for HBsAg ***(77,78)*** and one study analyzed two wild-caught, orphaned chimpanzees ***(79)*** to document that chHBV is distinct from all forms of hHBV. Only one study looked at tissues from animals that died in the wild and found a distinct chHBV in an east African chimpanzee ***(80)***.

The first observed case of confirmed hepatitis B in captive chimpanzees was reported in 1978 when several animals of a London Zoo breeding group showed clinical symptoms ***(81)***. The virus responsible for this infection was sequenced in 1988 and its sequence was 10% divergent from that of any human HBV sequence ***(82)***. At the time it was thought to resemble African hHBV because its serotype was identical to adw1. However, based on abundant HBV sequence data, it is now apparent that serotypes do not strictly correspond to genotypes, which is why all recently described chHBV can share serotype adw, despite having divergent sequences. Two HBV with typical sequences for gibbon HBV were found in two different captive chimpanzees, both of serotype ayw, and both very likely to have been infected by gibbons in captivity ***(83,84)***. There are now 11 published sequences of the complete chHBV genome derived from chimpanzees **(Table 4)**. These must represent a minimum of the HBV diversity existing in the wild chimpanzee populations.

These chHBV sequences have been used repeatedly to attempt reconstructing the phylogeny of primate HBV. Initial analysis of genomic sequences of various viral strains used only short sequences (usually parts of the S-gene), and did not take into account the frequent recombination occurring between HB viruses. A more recent analysis by Fares and Holmes ***(85)*** is based on total HBV genome sequence but

excluded obviously recombinant sequences as well as all segments with overlapping reading frames for methodological reasons. While it is now clear that there are several specific chHBV strains, the precise history of direction of infection, human to nonhuman primates, or nonhuman primates to human, or complex combinations of both, cannot be clearly deduced. Thus a simple explanation for presence of hHBV in nonhuman primates by human to animal infection is not possible. The question arises whether some of the nonhuman primate species could represent reservoirs, whereas others may have been infected by another animal species (e.g., gibbons from orangutan, or vice versa). If chimpanzees are a reservoir, then this would beg the question why chHBV diversity appears so much more restricted than that of hHBV. The curious case of a very divergent HBV in woolly monkeys is puzzling and its relationship to the most divergent strain of hHBV, hHBV-F, is a total mystery. To our knowledge, 9 of 16 animals positive for wmHBV were housed in the same facility in a North American zoo ***(86)***. There are no prevalence studies in or near natural woolly monkey habitat and no neotropical primates have tested positive for HBV in any other facility. The existence of a unique strain of HBV combined with the lack of African strains in the New World is puzzling and raises questions about why "African" hHBV was not imported despite the forced movement of millions of humans during the slave trade.

Several factors may explain why we still lack a clear reconstruction of HBV evolution. The first is that HBV mutates under selection pressure, so the mutation rate is difficult to predict. This has been best demonstrated in humans who developed recurrent HBV after orthotopic liver transplantation and while receiving regular doses of anti-HBV-immunoglobulin. Ghany et al. ***(87)*** sequenced the HBV genome before and after transplantation and demonstrated a change in "a" determinant in 50% of those expressing subtype adw2 and of the "S" gene in 85% posttransplantation. Thus the mutation rate of hHBV is subject to strong fluctuations and this variable has yet to be incorporated into phylogenetic analyses. Second, there appear to be recombination hot-spots along the HBV genome. Bowyer and Sim ***(88)*** found in their analysis of 65 whole HBV genome sequences that at least 14 carry clear signs of recombination ***(89)***. They concluded that the HBV genome consists of alternating conserved and highly variable domains, with the core region apparently most involved in recombination. The persistence of HBV in the host genome long after the acute infection subsides undoubtedly provides ample opportunity for viral recombination during subsequent infections. It would be important to study the precise nature, number, and degree of variation of persistent HBV genomes in host cells of chronic carriers, as has been done for HIV ***(90)***.

We are left with several hypotheses to explain the origin of HBV, and the nature of the correct hypothesis is highly relevant to the adequacy of the use of the chimpanzee model for research on HBV. If one examines the nonoverlapping areas of genomes to minimize effects of functional constraints on sequence evolution, and assumes a constant mutation rate, it appears that HBV arose within the past 6000 yr and that, because of similarities between ape and human HBV, both groups were infected at approximately the same time. However, if one makes comparisons of complete genome sequences ***(91)*** using calculated mutation rates (based on intra-host HBV evolution), it appears that the virus may be ancient. In support of this is the observation that hHBV-F

is found primarily in Polynesians and 70% of Amazon Indians ***(92)***, where different strains persist in geographically isolated populations. This indicates a more ancient divergence of human HBV (>15,000 yr) because of lack of substantial contact in intervening years. Alternatively, there could have been contact between Polynesia and the New World within the past 2000 yr. Another observation in favor of prolonged coevolution between HBV and its host is that there are geographic similarities of chHBV based on mt DNA sequence comparisons and HBV data ***(25)***; however, the generally higher genetic variability of chimpanzees as compared to humans is not reflected in the variability of their HBV. This latter point may be due to a strong bias in sample size for human viruses, leaving us with a strong underestimation of the real diversity of HBV in wild chimpanzees. The origin of HBV could be reconstructed if substitution rates were constant and recombination patterns known. To complicate matters further, the mutation rate of chHBV has not been calculated, and could differ from that of hHBV. Taken together, it is currently not possible to make a definitive statement about the origin of HBV because there are inconsistencies with both current theories. It is likely that HBV has a complex evolutionary history that may include recurrent cross-species infection between humans and apes, and periods of accelerated mutation rates in some of the host species. The consequences of the existence of chHBV for the use of the chimpanzee model for HBV infection cannot be safely determined at this point.

11. Differences in Host Genetics Between Chimpanzees and Humans

At the genomic level, humans and chimpanzees share more than 98% identity ***(93)***. Despite this high level of genetic similarity, chimpanzees have obvious phenotypic and significant functional differences, as exemplified by differing responses to HBV and other viruses. Recently, several groups have reported specific genetic differences between humans and chimpanzees. It is perhaps not surprising that several of the known genetic differences between humans and chimpanzees are connected to the immune system. It has been reported that the normal range of peripheral leukocytes in chimpanzees in captivity is 60% higher than in humans ***(94)***, but because the increase is in the number of polymorphonuclear leukocytes, this has not been considered to be the mechanism for differences in susceptibility to viral infections.

The primary host defense to viral infection is recognition of viruses by the major histocompatibility class (MHC) system. Although the functional orthologs in the MHC I genes of the human (HLA-A through G) have been described and found to be similar in chimpanzees ***(95,96)***, there are also notable differences. Chimpanzees have a much reduced repertoire of MHC I A alleles, lacking alleles falling into one of the two class I A lineages (based on exon 2 and 3 sequence data) ***(97–99)***. Furthermore, a recent study of intronic variation has demonstrated that chimpanzees must have undergone a selective sweep causing a marked reduction of gene repertoire at all three MHC Ia loci (A, B, and C) ***(100)***. Despite this loss of numerous ancient MHC I lineages and at least two MHC II lineages, chimpanzees still harbor more variation at exon 2 and 3 sequences (coding for the binding region of the molecule) of their class I B and C loci ***(101)***. Because the immediate response in both humans and chimpanzees is a strong, polyclonal CTL response to envelope, capsid, and polymerase proteins of HBV, and in

humans this response has been shown to be restricted to certain HLA alleles (HLA-A2, HLA-A3, HLA-B7 supertypes), this difference may be important to the outcome of HBV infection.

Despite this observation, it has been shown that infection of two chimpanzees with HBV with a terminally redundant copy of the HBV genome transgenically expressed in mice resulted in acute but self-limited HBV infection with identical CTL responses as humans ***(100)***. In fact, one chimpanzee responded to HLA-A2 supertype-restricted CTL epitopes (Env 183–191 and 335–343) and Pol (575–583) regions. This indicates that chimpanzees can mount effective responses to HLA-A2 and HLA-B7 supertype epitopes. Studies of MHC recognition of HIV by chimpanzees and human nonprogressors have shown that MHC molecules recognizing identical HIV epitopes belonged to very different allele lineages ***(102)***. The lack of certain MHC lineages may be protective, as several viruses have been shown to exploit host MHC molecules for immune subversion (e.g., *nef* gene in HIV ***[103]***).

Perhaps more relevant to interaction of HBV, chimpanzees have a nonclassical MHC I gene *Patr-AL*, which is lacking in humans ***(104)***. The rapid evolution documented for the KIR (killing inhibitory receptors) genes of ape and human natural killer (NK) cells has generated unique sets of genes in chimpanzees and copy number polymorphisms in humans and chimpanzees ***(105)***. KIRs have lectin-like domains that may interact with carbohydrate moieties on MHC molecules of target cells. The inactivating mutation of the single copy gene for the sialic acid modifying enzyme CMP–*N*-acetylneuraminic acid hydroxylase (CMAH) uniquely in humans has caused a change in the terminal cell surface glycosylation of virtually every cell. Humans have been shown to lack a form of sialic acid (*N*-glycolylneuraminic acid) otherwise common in mammals including chimpanzees ***(106)***. The biological consequences of this loss of function mutation are still being investigated. One known consequence is a change in macrophage biology due to a dramatic change in ligand density for sialoadhesin (Siglec 1) ***(107)***. These results provide potential for differences in how the immune system deals with HBV and other infections.

Another human-specific loss of function mutation is found for the gene coding for Siglec-L1, which in humans has lost the capacity to bind to sialic acid, while it retains this capacity in chimpanzees ***(108)***. The existence of differences between chimpanzee and human immune systems must obviously be kept in mind when contemplating the further use of chimpanzees as models for human disease.

12. Viral Envelopes and Differences in Host Cell Surfaces Between Humans and Chimpanzees

HBV is an enveloped virus and thus viral particles carry large numbers of cell surface glycoconjugates: proteins or lipids decorated with carbohydrate chains (glycans). The vast majority of these glycans are capped by sialic acid molecules. Potential roles of sialic acids in natural immunity have been proposed ***(109)***. The potential role of glycosylation variants in vaccines has not been sufficiently addressed, especially with regard to the glycan structure and composition produced in different recombinant

expression systems. Yeast, insect, and mammalian cells have drastically different *N*-glycans, and yeast high-mannose glycans have previously compromised vaccine design (as demonstrated by the failed vaccine attempt targeted at HIV gp120). Furthermore, it has been shown that blocking the assembly of *N*-glycans on S proteins of HBV leads to the retention of viruses inside the host cell and prevents viral replication cycles ***(110)***. Because *N*-glycolylneuraminic acid is known to be antigenic in humans but not in chimpanzees, its presence on vaccines may alter the antigenicity, and thus the effectiveness, of the vaccine in these two closely related species.

In this context, it is interesting that one inoculation attempt with woolly monkey HBV into chimpanzee produced only minimal infection ***(86)***. The enveloped HBV from the New World woolly monkey must have carried the strongly antigenic α Gal epitope. α 1–3 linked galactose (to another galactose) a structure that is completely absent in old world primates (Catarrhines). Old world primates combine the lack of this α Gal with high titers of natural antibody (IgG) against this carbohydrate epitope found in most other mammals, and it has been suggested that this forms an efficient barrier to infection by enveloped viruses from other species ***(111)***.

Unfortunately, the nature of the receptor(s) used by HBV remains elusive. Atkins et al. showed that glycosaminoglycans (proteoglycans) influence HBV liver and leukocyte interactions ***(112)***. Budkowska et al. ***(113)*** found a soluble HBV binding factor in human serum. It is a glycoprotein and binding can be decreased more than fourfold by coincubation with wheat germ agglutinin and *Helix pomatia* (*N*-acetyl-b-D-glucosamine and to *N*-acetylgalatosamine residues) but is not affected by incubation with peanut agglutinin (Galbeta1-3GalNAc) or concanavalin A (high mannose type glycans). It was purified after incubation of human serum with pre-S1 and pre-S2-specific monoclonal antibodies but demonstrates no binding to HBV S protein. The fact that this soluble protein interacts with pre-S epitopes is interesting because HBV appears to bind to cells via this region ***(114)***. However, as long as the receptor(s) used by HBV remain unknown, it is impossible to speculate on similarity of receptors in chimpanzees, or on the impact of the glycosylation differences on cell surfaces.

13. Conclusions

The chimpanzee model has been crucial for vaccine development and for improving safety of blood products. Despite the large amount of work carried out on HBV in chimpanzees and the impressive numbers of animals used for infection and vaccine work, we are left with a confusing picture of long-term effects of infection with human HBV in chimpanzees, and a nearly complete ignorance of the native chHBV infection in wild chimpanzee populations. Although chimpanzees were important in the development and testing of the currently used HBV vaccines, it is questionable whether chimpanzees will be of much help in the development of therapeutic DNA vaccines or drugs for treatment of chronic HBV infection. There is an urgent need for a concerted effort to identify the surviving chimpanzees chronically infected with hHBV or chHBV. Only a careful longitudinal study of this very small group of animals would allow determination of whether the chronic carrier state is really comparable between chimpanzees and

humans. The small number of chimpanzees that develop chronic infection, in addition to ethical issues surrounding use of primates for research ***(115)***, make it likely that future studies will have to be carried out in naturally infected human populations.

Several other human viruses have been documented to have counterparts in wild chimpanzees, including HIV1/SIVcpz and HTLV1/STLV1, Ebola, TT, Spuma, Kaposi sarcoma herpes, and monkeypox viruses. Efforts are ongoing to document the epidemiology of these agents in wild ape populations in Africa. Concerted efforts to obtain good quality noninvasive samples from field research sites across Africa could provide valuable opportunities. Fecal samples, urine samples, and samples of chewed fruit ("wadges" containing saliva and many buccal cells) can easily be collected in large numbers at field sites where wild animals have been habituated to human observers (at least 10 such sites exist across Africa) and samples can be obtained even from nonhabituated populations (Gagneux, *personal experience*, ***116***). Considering the endangered status of most wild chimpanzee populations in Africa, which face human encroachment on their habitats, habitat destruction, and most of all growing hunting pressure by humans, it may very well be the last opportunity to document the epidemiology of such viruses in wild chimpanzee populations, and to relate this epidemiology to their human counterparts.

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