Perspectives on Hepatitis B Studies with Chimpanzees

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Abstract

Chimpanzees have been shown to be exquisitely susceptible to human hepatitis viruses, without themselves developing clinical illness, thus providing an important model for studies on these agents. Chimpanzees have contributed substantially to human welfare by making possible the development of hepatitis B vaccines, which now prevent development of cirrhosis and hepatocellular carcinoma in millions of people. They have provided a means to evaluate the efficacy of virus inactivation strategies, which have made blood derivatives formerly contaminated with blood-borne viruses (hepatitis B, C, and human immunodeficiency viruses) safe with respect to their transmission. In exchange for these contributions, humans owe chimpanzees lifelong retirement in sanctuaries that offer socialization and environmental enrichment.

Key Words: animal welfare; blood safety; chimpanzees; hepatitis B virus; HBV vaccine

Establishment of the Model

The discovery of a hepatitis-specific antigen, originally called the SH antigen but now called HBsAg (Prince 1968), was followed by a search for this antigen in chimpanzee serum collections. The rationale was that because humans in Africa were known to have a high (generally about 10%) rate of positivity to the hepatitis B surface antigen (HBsAg), chimpanzees originating in Africa might also have similar high rates of infection. This hypothesis was confirmed when HBsAg was found in the sera of many chimpanzees (Eichberg and Kalter 1980; Lichter 1969; Maynard et al. 1972; Prince 1972a). The possibility that these infections originated in the wild was considered but could not be proven until very recently (Hu et al. 2000; MacDonald et al. 2000; Takahashi et al. 2000).

Prince (1972a,b) reported the first documentation of de novo infections of chimpanzees by hepatitis B virus (HBV). Inoculation of chimpanzee plasma found to be spontaneously positive for HBsAg produced hepatitis, HBs antigenemia, and anti-HBs seroconversion in each of six chimpanzees inoculated. That this susceptibility applied also to human HBV inocula was shown by the demonstration of transient antigenemia and anti-HBs seroconversion in two chimpanzees that received human inocula (Maynard et al. 1972). Desmyter et al. (1973) reported similar results in two of five inoculated animals and Barker et al. (1973), in five of six inoculated animals. The studies described above clearly established chimpanzees as a useful animal model for HBV, making possible the studies reviewed in this article.

Biological Studies

Before embarking on development of vaccines and therapies, virologists must characterize the biological properties of target viruses. Examples of such studies carried out in chimpanzees follow.

Barker et al. (1975) and Tabor et al. (1983) prepared pools of infectious virus corresponding to the known subtypes of HBV. They titrated these pools by inoculation of serial dilutions into chimpanzees and determined that the patterns of infection closely resembled those seen in humans. These studies were an essential prerequisite for subsequent vaccine development and evaluation in the chimpanzee model.

Thomssen et al. (1977) provided evidence that the 42-nm “Dane particles” were the infectious virion of HBV. Shikata et al. (1980) characterized the two types of infection that occur in both humans and chimpanzees—the acute self-limited form and the form that evolves into the chronic carrier state. Brotman et al. (1983) showed that hepatitis C infection markedly inhibits hepatitis B infection in chimpanzees. This finding had important implications for studies on inactivation of viruses in pooled plasma products inasmuch as both viruses could be present in these animals. Tabor et al. (1983) showed that there was an inverse relation between the titer of HBV inoculation and incubation periods before the appearance of HBsAg. This knowledge was subsequently used (Prince et al. 1987) to monitor removal and inactivation of virus during the production of a factor IX preparation. Prince
panzees, initially involved the intravenous inoculation of one clinical trials. More, they provided background for the selection of dose to determine whether infectivity occurred. The evaluation of into two chimpanzees (and later one) with 6 mo of follow up live HBV. Thus, for many years, all manufacturers of HBV vaccine were required to submit their production batches, to chimpanzee safety tests. These tests, performed in more than 100 chimpanzees, initially involved the intravenous inoculation of one and 10 clinical vaccine doses of unadjuvanted vaccine each into two chimpanzees (and later one) with 6 mo of follow up to determine whether infectivity occurred. The evaluation of safety tests is described in detail in Berthelot et al. (1984). It is a tribute to the safety of the manufacturing process, and to the skill of the manufacturers, that none of these tests revealed infectivity.

Some plasma-derived vaccines contained the hepatitis B e antigen (HBeAg), an epitope on the core/core protein. To investigate its importance, Stephan et al. (1984) prepared an immunoglobulin-containing antibody to the hepatitis core antigen (anti-HBc) alone, or anti-HBe + anti-HBc. The latter, but not the former, markedly prolonged the incubation period of HBV in chimpanzee experiments. The explanation of this provocative finding remains obscure to the present time.

In recent years, a new approach to immunization, the use of “naked” DNA vaccines, has generated great interest. Such vaccines induce cell-mediated immunity and are attractive in principle as approaches to multivalent immunogens able to protect against various pathogens. Inasmuch as HBV vaccines are given to infants in Asia at birth because of frequent mother-to-baby transmission, we immunized newborn chimpanzees (Prince et al. 1997). Protection was observed against a challenge given at the age of 6 mo. However, a subsequent study revealed that protection did not result when the challenge was on the day of birth (Prince et al. unpublished). Additional improvement in the immunizing strategy is required to induce immunity with DNA-based vaccines that will be sufficiently rapid for this application.

Vaccine Studies

Trepo et al. (1975) immunized six chimpanzees with HBsAg, and all developed high titers of anti-HBs and delayed hypersensitivity skin reactions. No such reactions were seen in healthy chronic HBV carrier chimpanzees. Prince et al. (1978) described the evaluation of candidate HBV vaccine safety and immunogenicity in chimpanzees. These and subsequent studies (Lelie et al. 1987; Prince et al. 1981) provided some assurance that the vaccines, which were then made from the infectious plasma of infected human chronic carriers, were actually free from infectious virus. Furthermore, they provided background for the selection of dose and the schedule of vaccination to be used in subsequent clinical trials.

Because no in vitro assay was available for detection of infectious HBV, use of the chimpanzee was the only way to ensure that lapses of Good Manufacturing Practice did not result in batches of plasma-derived HBV vaccine containing live HBV. Thus, for many years, all manufacturers of HBV vaccine were required to submit their production batches, which consisted of up to a million doses, to chimpanzee safety tests. These tests, performed in more than 100 chimpanzees, initially involved the intravenous inoculation of one and 10 clinical vaccine doses of unadjuvanted vaccine each into two chimpanzees (and later one) with 6 mo of follow up to determine whether infectivity occurred. The evaluation of safety tests is described in detail in Berthelot et al. (1984). It is a tribute to the safety of the manufacturing process, and to the skill of the manufacturers, that none of these tests revealed infectivity.

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Evaluation of the Inactivation of HBV and HCV for Manufacture of Virus-free Plasma Derivatives

The Biotest Company was the first manufacturer of human plasma derivatives to recognize the high-level risk of transmission of hepatitis viruses by these blood products. Beginning in 1970, Wolfgang Stephan and his colleagues began to evaluate the suitability of various strategies for inactivation of viruses without loss of biological activity of the therapeutic blood proteins. The availability of the chimpanzee model for infection with human hepatitis viruses permitted the evaluation of the efficacy of these strategies. In 1980, the first of many reports of the evaluation of such strategies appeared (Prince et al. 1980). In this study, we evaluated the safety of a factor IX preparation made with and without “cold sterilization” by β-propiolactone and ultraviolet irradiation. The untreated product infected four of four chimpanzees whereas the treated product was not infectious. In addition, we contaminated a factor IX preparation with hepatitis B virus. Cold sterilization reduced the infectivity to borderline levels (one of four chimpanzees was infected). Stephan and colleagues (1981) subsequently estimated that the procedure reduced the titer of HBV by approximately 106-fold.

An alternative approach for sterilization of blood derivatives was introduced by Prince et al. (1984). We assessed the efficacy of using various solvent/detergent combinations for inactivation of blood-borne viruses. The combination of Tween 80 and ether, classically used by virologists to determine whether a virus isolate is lipid enveloped, was found to inactivate at least 106 chimpanzee infectious doses (estimated to infect 50% of the chimpanzees [CID50]) of HBV, and >104 CID50 of hepatitis C virus (HCV1), without major reduction in the biological activity of factor VIII. We (Prince et al. 1986) later reported the evaluation of a solvent/detergent procedure that was more compatible with use in a manufacturing environment—the combination of the nonflammable solvent tri(n-butyl) phosphate and the detergent sodium cholate. Two chimpanzees inoculated with 106 CID50 of either HBV or HCV treated in this manner remained free from infection after 9 mo of follow up. The process was further validated by the observation that 80 mL of factor VIII from each of 13 lots of factor VIII, prepared by five manufacturers and inoculated into two chimpanzees, was not infectious. In all of these studies, because sensitive serological procedures were not yet available for HCV, the sensitivity to infection of the inoculated chimpanzees was confirmed by inoculation of untreated virus. The development of virus-free labile blood derivatives and the role of chimpanzees in the validation of these procedures have been reviewed (Prince et al. 1987).
Infection of Chimpanzees in the Wild

HBV infection of chimpanzees in captivity was observed by many investigators. Until recently, however, there has been no way to determine whether these infections were acquired in the wild or from humans. It has been speculated that chimpanzee exporters in West Africa were giving the animals injections of human blood to provide passive immunity and prevent illness during shipment.

Recently, the sequencing of DNA from HBV genomes, amplified by polymerase chain reaction, has provided an exquisitely sensitive and accurate way to genotype isolates. This technology has led to the division of human HBV strains into at least six genotypes. Sequencing of an HBV isolate from an infected chimpanzee in the London Zoo revealed this to be a unique nonhuman genotype (Vaudin et al. 1988). Identical sequences have been reported recently in other HBV-infected chimpanzees (Hu et al. 2000; MacDonald et al. 2000; Takahashi et al. 2000). Thus, it is now clear that a distinct chimpanzee HBV has co-evolved with chimpanzees.

Ethical Considerations

In recent years, there has been an increasing improvement in our understanding of the near-human nature of chimpanzees. Foremost among those responsible for this knowledge is Jane Goodall, whose observations of the behavior of chimpanzees in the wild dramatically increased our awareness of this species and its similarities to humans. Because chimpanzees began to be used in medical research due to their biological similarities to humans, those investigators who took the opportunity to get to know their research subjects became aware of the needs of these animals.

In the early days of the use of chimpanzees for HBV research, the first large-scale application of the chimpanzee model in biomedical research, chimpanzees were acquired from importers who in turn acquired them from exporters in West Africa (primarily Sierra Leone). Numerous observers attested to the fact that the export of one chimpanzee was associated with the death of 10 others, because infant chimpanzees were captured by shooting adults and because the mortality of the stressed infants was very high. It has become obvious that chimpanzees in the wild are an “endangered” species due to the expansion of logging, large-scale hunting, and agriculture into their forest habitats. Since the mid-1980s, the US Fish and Wildlife Service and the Convention on International Trade in Endangered Species of Wild Fauna and Flora has prohibited importation of wild caught chimpanzees. Future supplies of chimpanzees for medical research must therefore be obtained from existing colonies or by breeding.

Observation of chimpanzees in medical research laboratories in the 1970s, and in some cases up until this time, revealed animals caged mostly alone and often in cages with floor areas of only 5 x 7 ft, an inadequate height for an adult to stand up straight. Not surprisingly, the chimpanzees held under these conditions were usually depressed, as could be seen from their compulsive repetitive movements such as “rocking.”

In 1974, having experienced the conditions described above in US laboratories, we decided to establish a chimpanzee research laboratory in Liberia. Our motivation was as follows: (1) We would be able to capture chimpanzees humanely and with minimal mortality using a specially trained team of hunters equipped with anesthetic dart rifles and working in a forest region that was in the process of being logged and converted into farmland. Chimpanzees in such areas are endangered because they will raid the fields of farmers, who will then shoot them to protect their farms (Prince 1981). (2) Because labor and land in Africa are inexpensive and the climate is pleasant year-round, it would be possible to make large outdoor enclosures and provide the opportunity for group housing, socialization, exercise, and environmental enrichment (Prince et al. 1989, 1990). (3) When animals are no longer involved in research, they could be retired into wild or semiwild sanctuaries. Initially, we hoped to be able to retire chimpanzees into national parks. However, this plan proved to be impractical because we recognized that the chimpanzees in the wild might not accept the “invaders,” and that chimpanzees who have known humans and have lost their fear of them would probably harass villagers living adjacent to parks. We therefore embarked on retiring groups of 15 to 20 animals to 10- to 30-acre islands in rivers close to our laboratory.

Shortly after establishing our colony in Liberia, we decided that our existing cages were too small for group housing and did not provide adequate opportunity for exercise. Because considerable time would have been required to construct suitable cages, we transferred the animals outdoors with 3- to 6-meter collar chains that were connected to platforms or jungle gyms on concrete pads, or with steel collars that encircled tree trunks (van den Ende et al. 1980). The chimpanzees, all less than 6 yr old, were thus afforded unlimited opportunity for play.

In 1981, the National Institutes of Health sponsored a meeting on alternatives to the use of animals in biomedical research in which one of us (A. M. P.) was asked to address the use of chimpanzees (Prince 1981). There appeared to be no alternative to the use of chimpanzees in such areas as hepatitis B vaccine development, evaluation of methods to eliminate viruses from plasma derivatives, research on non-A, non-B hepatitis, or putative human tumor viruses. However, the following declarations contained in a “Chimpanzee Bill of Rights” were proposed to address the ethical issues. (1) Chimpanzees must be captured in a humane manner. Today this would be changed to “Chimpanzees cannot be captured in the wild.” (2) Chimpanzees must be maintained under humane conditions that satisfy their special needs. This provision implies adequate space, social interaction with companions, and enrichment to minimize boredom. (3) Institutions sponsoring and carrying out chimpanzee research are responsible for the lifetime care of these animals under semi-free-ranging sanctuary conditions, if at all possible. These
three ethical considerations and corresponding statements have been expanded and detailed in additional publications (Prince 1993; Prince et al. 1989, 1990).

References


