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RAPID COMMUNICATIONS

LACK OF SUSCEPTIBILITY OF BABOONS TO INFECTION WITH HEPATITIS B VIRUS

MARIAN G. MICHAELS,[1,2] ROBERT LANFORD,[3] ANTHONY J. DEMETRIS,[4] DEBORAH CHAVEZ,[3]
KATHLEEN BRASKY,[5] JOHN FUNG,[4] AND THOMAS E. STARZL[4]

Departments of Pediatrics and Transplantation, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania,
and Departments of Virology and Immunology, and Laboratory Medicine, Southwest Foundation for Biomedical Research,
San Antonio, Texas

Historically, hepatitis B virus (HBV) has been considered species specific and unable to infect baboons. Based on this premise, two patients with HBV end-stage liver disease underwent baboon liver xenotransplantation. To study whether baboons are susceptible to HBV infection, four baboons (two receiving immunosuppressive therapy) were inoculated with HBV. Animals were followed for 6 months: clinical examinations and biochemical studies were normal, hepatitis B surface antigen and hepatitis B core antigen staining of biopsies was negative, and HBV serology remained negative. HBV polymerase chain reaction was transiently positive in one animal, which most likely reflects the initial inoculation. This pilot study corroborates historical evidence and beliefs that baboons are resistant to HBV.

Between the 1940s and 1960s, investigators sought a nonhuman primate model for hepatitis B by inoculating material from patients with clinical hepatitis into various primate species (reviewed in 1 and 2). Papio sp (baboons) included in this group developed neither clinical nor biochemical signs of infection. The sophistication to detect hepatitis B virus (HBV*) antigen (hepatitis B surface antigen [HBsAg]) was not available for these studies. Consequently, asymptomatic infections could be missed. Previously infected animals would not have been detected and their resistance to infection would have been misinterpreted. When HBV antigens and antibodies were identified, a prevalence study including 99 baboons was performed; all baboons were seronegative for

HBV (3). The unsuccessful attempts to induce hepatitis by inoculation of infectious material from humans with hepatitis coupled with the lack of detectable HBV antigen in this large survey of baboons (2, 3) led to the conclusion that baboons are resistant to infection with HBV. However, subsequent serologic surveys reported conflicting results, with anti-HBsAg found in 4 of 7 (4) and 23 of 65 (5) baboons. HBV screening before two liver xenotransplantations at the University of Pittsburgh found 1 of 31 baboons to have discordant results for anti-HBsAg when tested at two different laboratories (6). Accordingly, there has been uncertainty about whether baboons are resistant to HBV infection. None of these studies used molecular techniques to examine the presence of HBV infection.

Two baboon to human liver xenotransplantations were performed at the University of Pittsburgh on patients suffering from end-stage liver disease secondary to chronic HBV infection. The transplant recipients survived 70 and 25 days, respectively. Liver biopsy samples and autopsy material of the xenograft livers did not show histologic evidence of HBV infection and immunohistochemical staining for HBsAg and hepatitis B core antigen (HBcAg) was negative (7). Polymerase chain reaction (PCR) studies were negative for HBV in 4 of 5 samples from the xenograft livers (8). The one positive sample was at the lower limits of detection for the assay and was negative on subsequent testing. The positive test result on this liver tissue may have reflected the presence of the patient's peripheral blood lymphocytes (which were assayed separately and found to be positive) (8). The current pilot study was undertaken to further examine the susceptibility of baboons to HBV infection using currently available molecular methods.

METHODS

Four baboons were inoculated intravenously with 1 ml of a dilution of infectious serum containing $10^{4.9}$ chimpanzee infectious doses of an NIH standardized dose (gift of Dr. Robert H. Purcell, National Institutes of Allergy and Immunology Division, NIH). The specific infectivity of this inoculum had been determined previously in HBV infectivity studies with chimpanzees (9). Two of the four baboons were immunosuppressed with oral tacrolimus (FK506), 12 mg/kg/day (supplied by Fujisawa Pharmaceutical Co., Japan). All studies were approved by the Animal Research Committee of Southwest

[1] Department of Pediatrics, University of Pittsburgh, School of Medicine.

[2] Address correspondence to: Marian G. Michaels, MD, Children's Hospital of Pittsburgh, Infectious Diseases, 3705 Fifth Avenue at DeSoto Street, Pittsburgh, PA 15213.

[3] Department of Virology and Immunology, Southwest Foundation for Biomedical Research.

[4] Department of Transplantation, University of Pittsburgh, School of Medicine.

[5] Department of Laboratory Medicine, Southwest Foundation for Biomedical Research.

* Abbreviations: HBV, hepatitis B virus; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B early antigen; HBsAg, hepatitis B surface antigen; PCR, polymerase chain reaction.

Foundation for Biomedical Research. Animals were fed and watered ad libitum and were housed in quarantine facilities. Procedures were performed using intramuscular ketamine (10 mg/kg) and intramuscular diazepam (5 mg) for sedation. Baboons were followed and assessed daily for clinical illness. Blood specimens were obtained prior to inoculation and then weekly for a complete blood count, liver enzyme profile (total bilirubin, aspartate and alanine aminotransferases, γ-glutamyltransferase), and tacrolimus levels in the animals receiving immunosuppression.

HBV studies. Prior to HBV inoculation, serology was performed for HBsAg, anti-HBsAg, anti-HBcAg, hepatitis B early antigen (HBeAg), and anti-HBeAg. In addition, total anti-hepatitis A virus was evaluated along with antibody against hepatitis C virus and hepatitis delta virus. Repeat HBV serology was performed weekly (HBsAg, anti-HBsAg, anti-HBcAg). Weekly serum samples were analyzed by PCR, as described previously (9), to determine whether the baboon might support a low level of HBV replication not detected by previous methods.

Liver biopsies. Percutaneous liver biopsies were performed prior to HBV inoculation and at 2-month intervals. The tissue specimens were routinely fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. For detection of HBcAg and HBsAg with immunocytochemistry, a standard avidin/biotin complex was used with polyclonal primary reagents (anti-HBc, rabbit polyclonal, anti-HBs, goat polyclonal; DAKO) for 1 hr, followed by the appropriate biotinylated secondary antibody, avidin/biotin complex, and color development. An irrelevant primary antibody was substituted for the primary antibody in the negative controls, and known positive tissues were included as positive controls. No other special stains were routinely performed because the liver biopsy architecture was normal at all times.

RESULTS and DISCUSSION

All animals remained clinically healthy during the 6-month study. Animals receiving tacrolimus had average levels of 0.3 and 0.5 ng/ml, respectively (target level: 0.3–0.7 ng/ml). There was no evidence of hepatocellular dysfunction nor hematologic abnormalities. HBV DNA and serologic studies likewise remained negative on all animals. Liver biopsy specimens showed normal hepatic architecture and stains for HBcAg and HBsAg were negative. Serum PCR results were weakly positive on one animal (not immunosuppressed) during weeks 2–5. Weekly samples, from weeks 6–9, following inoculation were all negative on this animal. Repeat PCR of all samples subsequently failed to detect positive reactions on the same sera. The initial positive findings were at the limits of detection by PCR and may represent transient persistence of the inoculum. Delayed clearance of HBV DNA, as detected by PCR, was previously reported in macaques (10).

Although the sample size of this current study is limited, the inability to detect HBV infection by PCR, liver biopsy evaluation, and serology after inoculation of two immunocompetent baboons and two other immunosuppressed animals supports the contention that baboons are resistant to HBV infection. While consistent with classical World Health Organization studies (1) and reports by Deinhardt (2), this conclusion is at variance with two reports of seropositivity

36% (4) and 57% (5) of surveyed baboons. Previously, we found only 1 of 31 (3%) of baboons to be anti-HBsAg positive (6) from the same colony which 14 years earlier had detected anti-HBsAg in 51.9% of wild caught baboons and 27.6% of colony-raised animals (3). Testing of the one seropositive animal by a second laboratory yielded negative results (5). Thus, positive serologic tests may have been falsely positive, or represent cross-reacting antibody to a related virus. Alternatively, the current investigation may have failed to demonstrate transmission because of the small number of subjects. The dose of tacrolimus used in the two immunosuppressed animals was based on previous studies of immunosuppression in baboons (11). However, lymphocyte studies were not performed to verify immunosuppression. Nonetheless, failure to detect even minimal viral replication using a highly sensitive PCR assay supports the premise that baboons are in fact resistant to HBV infection. This information will be valuable in planning treatment strategies involving the use of xenografts.

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