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Markedly prolonged incubation period of hepatitis B in a chimpanzee passively immunized with a human monoclonal antibody to the *a* determinant of hepatitis B surface antigen

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ABSTRACT **The protective efficacy of a human monoclonal antibody directed against the *a* determinant of hepatitis B virus (HBV) surface antigen was studied in a chimpanzee. A single high dose of 5 mg/kg (body weight) of monoclonal antibody SDZ OST 577 was intravenously administered to a chimpanzee, followed by intravenous challenge with $10^{3.5}$ chimpanzee infectious doses of a wild-type HBV, the MS-2 strain (*ayw* subtype). The passively acquired antibody to HBV surface antigen could be detected for 40 weeks. Serum HBV DNA tested by a "nested" polymerase chain reaction assay was negative through the 36th week after virus challenge but became positive by the 38th week. The chimpanzee subsequently developed acute hepatitis B ≈1 year after challenge. The nucleotide sequence of the *a* determinant of the surface gene of the replicated virus was identical with that of the inoculated wild-type virus. Thus, a human monoclonal antibody directed against the *a* determinant of HBV surface antigen delayed but did not prevent experimental infection of HBV and hepatitis in the chimpanzee. Our results indicate an incomplete ability of this antibody to protect against HBV infection *in vivo* after a single infusion.**

The major surface protein of the envelope of the hepatitis B virus (HBV) contains an antigenic determinant in a hydrophilic region, termed the *a* determinant, encompassed by codon positions 110–156 (1–7). The *a* determinant is believed to contain an essential epitope that reacts with protective or neutralizing antibodies to HBV irrespective of virus subtype (1–7). This has suggested the possibility of using monoclonal antibodies directed against the *a* determinant for the immunoprophylaxis or treatment of HBV infection and disease. Recently, one such antibody, human monoclonal antibody SDZ OST 577 (8, 9), was administered to a small number of orthotopic liver transplant patients who underwent transplantation for end-stage liver disease associated with chronic HBV infection in an attempt to prevent recurrence of hepatitis B (10). Although the results suggest that multiple doses of this monoclonal antibody could prevent recurrent hepatitis B in some liver transplant patients, the protective efficacy of the antibody has not been evaluated in a systematic manner. To determine whether monoclonal antibody SDZ OST 577 has protective activity against HBV infection *in vivo*, we administered a single high dose of the antibody to a chimpanzee and challenged the animal with the MS-2 strain of HBV (*ayw* subtype), a wild-type virus. The results demonstrated that, although the monoclonal antibody significantly prolonged the incubation period, it failed to protect the chimpanzee from HBV infection or hepatitis.

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MATERIALS AND METHODS

Animal. The chimpanzee used in this study, chimpanzee 1443, had never been inoculated with any HBV-containing materials previously and was seronegative for all HBV-associated serologic markers.

Human Anti-HBV Monoclonal Antibody (SDZ OST 577). A human monoclonal antibody, SDZ OST 577, directed against HBV surface antigen (HBsAg) was produced in a hybridoma cell line obtained by fusion of peripheral blood lymphocytes from a healthy adult male vaccinated against hepatitis B with Heptavax (Merck Sharp & Dohme) with the mouse–human heteromyeloma cell line SPAZ-4 (8, 9). The antibody is of the IgG1 subclass and has a λ light chain. It binds specifically and with high affinity [$K = 3.6 \times 10^9\ M^{-1}$] to HBsAg and it is at least 200-fold more active than commercial hepatitis B immune globulin (HBIG) (9). A SDZ OST 577 concentration of 1 μg/ml is equivalent to antibody to HBsAg (anti-HBs) at about 5000–10,000 RIA units/ml by RIA (highest dilution positive).

HBV Inoculum (MS-2 Strain). A challenge pool of wild-type HBV MS-2 (*ayw* subtype) (11, 12) was used for the experimental inoculation. The infectivity of this strain in chimpanzees has been determined (13, 14).

Experimental Administration of the Monoclonal Antibody and HBV Inoculation. The human anti-HBs monoclonal antibody SDZ OST 577 was administered intravenously to the chimpanzee at a dose of 5 mg/kg (body weight). Three days later when the antibody was believed to be equilibrated with the extravascular space, $10^{3.5}$ chimpanzee infectious doses of the challenge pool of HBV MS-2 was inoculated intravenously.

Serological and Biochemical Analyses of the Chimpanzee Serum. Serum samples taken from the chimpanzee were monitored for levels of SDZ OST 577, HBsAg, anti-HBs, antibody to hepatitis B core antigen (HBcAg; anti-HBc), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), HBV DNA, and liver enzymes such as alanine aminotransferase, isocitrate dehydrogenase, and γ-glutamyltransferase. Serum levels of SDZ OST 577 were quantified by a sandwich enzyme-linked immunoassay as described (9). Serological assays of the HBV antigens and antibodies were performed with commercial solid-phase radioimmunoassay kits (Abbott).

Immunofluorescence. HBcAg was sought in frozen sections of needle biopsies obtained from the chimpanzee at weeks 0, 1, 37, 40, 42, 44, and 46. Sections of liver tissue 4 μm thick were fixed in ice-cold acetone for 10 min, air-dried, and stored at −80°C. Sections were stained with tetramethylrhodamine-labeled human anti-HBc diluted in phosphate-

Abbreviations: HBV, hepatitis B virus; HBsAg, HBV surface antigen; HBIG, hepatitis B immune globulin; HBeAg, HBV e antigen; HBcAg, HBV core antigen.

buffered saline (PBS) containing 1% bovine serum albumin. Incubation was for 45 min at 37°C, followed by two 15-min washes in PBS. The immunofluorescence results were compared with a positive control consisting of a comparably prepared section of liver from a chimpanzee chronically infected with HBV.

Polymerase Chain Reaction (PCR). HBV DNA in serum and liver tissue was tested by a "nested" PCR assay (15). Serum DNA was extracted by a sodium hydroxide procedure (16). Liver tissue DNA was extracted essentially as described (17, 18). Serum (10 μl) or ≈1 mm^3 of the liver tissue was used for the reaction. Primers were synthesized to amplify a portion of the HBV surface (*S*) gene including the *a* determinant and were as follows: outer sense primer, 5′-CGAGAATTCAGAGTCTAGACTCGTGGTGGACTT-3′; outer antisense primer, 5′-TGAGGATCCTACGAACCACTGAACAAATGGCAC-3′; inner sense primer, 5′-CGCGAATTCTAGACTCGTGGTGGACTTCTCTCA-3′; inner antisense primer, 5′-CTAGGATCCACTGAACAAATGGCACTAGTAA-3′. (Underlined nucleotides represent restriction endonuclease recognition sites for *Eco*RI or *Bam*HI that were added to the primers.)

DNA Sequencing. Both strands of the DNA fragments of the HBV *S* gene recovered by PCR amplification of the challenge pool of HBV and from chimpanzee serum obtained at weeks 38, 41, 50, and 58 after the challenge were sequenced as described (15).

RESULTS

Pharmacokinetics of Human Monoclonal Antibody SDZ OST 577 in the Chimpanzee. Serum levels of the human anti-HBs monoclonal antibody SDZ OST 577 in chimpanzee 1443 were monitored for 4 months and the results are shown in Fig. 1. The serum level of SDZ OST 577 was 54.10 μg/ml on the 1st day after administration and declined to 0.74 μg/ml on the 113th day after administration, with a half-life of 16.7 days. The expected half-life of immunoglobulin in a human of equivalent body weight is estimated to be 15.7 days. Therefore, the half-life of SDZ OST 577 in the chimpanzee was considered to be within the normal range for native immunoglobulins. This indicates that the monoclonal antibody was not fragmented, seen as a foreign antigen by the chimpanzee, or consumed by the reaction with HBsAg in the animal.

Course of HBV Infection in the Chimpanzee. The course of HBV infection in chimpanzee 1443 after administration of the human anti-HBs monoclonal antibody and challenge with HBV is shown in Fig. 2. Serum anti-HBs became positive on the 1st day after the injection of SDZ OST 577 and was detectable for 40 weeks. Serum HBV DNA tested by the nested primer-PCR assay remained negative through the 36th week after challenge with HBV and then became positive during the 38th week, 2 weeks before the disappearance of passively acquired serum anti-HBs. The week after the disappearance of serum anti-HBs, during the 41st week, serum HBsAg became positive and the chimpanzee subsequently developed acute hepatitis with elevated serum levels of liver enzymes and typical profiles of serum HBeAg, anti-HBe, anti-HBc, and finally, actively acquired anti-HBs. Since the expected incubation period (to appearance of HBsAg) of the MS-2 strain in chimpanzees at the dose used in this experiment was 6–11 weeks (median, ≈8 weeks) and the longest incubation period, at the end-point of titration, has been reported to be 15 weeks (13, 14), it was thus apparent that a single high dose of the monoclonal antibody significantly prolonged the incubation period of HBV infection and hepatitis in this study. However, it was also clear that the antibody failed to protect the chimpanzee from HBV infection and hepatitis.

Nucleotide Sequence of the *a* Determinant of the *S* Gene of HBV (MS-2 Strain) and of HBV Recovered from the Chimpanzee. To determine whether the HBV recovered from the chimpanzee had developed *S* gene mutations during the long incubation period, we determined the nucleotide sequence from nucleotide positions 484 to 636 [according to the designation by Tiollais *et al.* (19)], corresponding to *S* gene codon positions 110–160 of the challenge virus and the recovered virus. The results are shown in Fig. 3. The MS-2 strain of HBV contained the amino acid residue isoleucine at codon position 127, which was different from the consensus sequence (Proline) of subtype *ayw* (20–26), but it was otherwise a typical wild-type virus. HBV DNA recovered from the serum of the chimpanzee at four time points during the infection—i.e., the 38th week (before the disappearance of the passively acquired serum anti-HBs), 41st week (appearance of serum HBsAg), 50th week (serum HBeAg-positive phase before the elevation of liver enzymes), and 58th week (serum anti-HBe-positive phase after the decline of liver enzymes)—all had nucleotide sequences identical with the inoculated MS-2 strain. Results of the direct sequencing of PCR-amplified HBV DNA fragments did not demonstrate any evidence for genetic heterogeneity in this gene region.

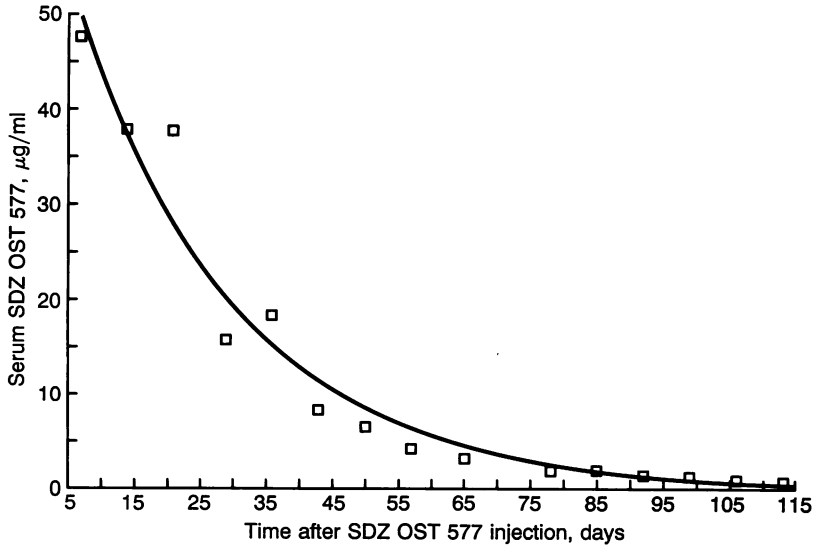

FIG. 1. Serum levels of SDZ OST 577 in chimpanzee 1443.

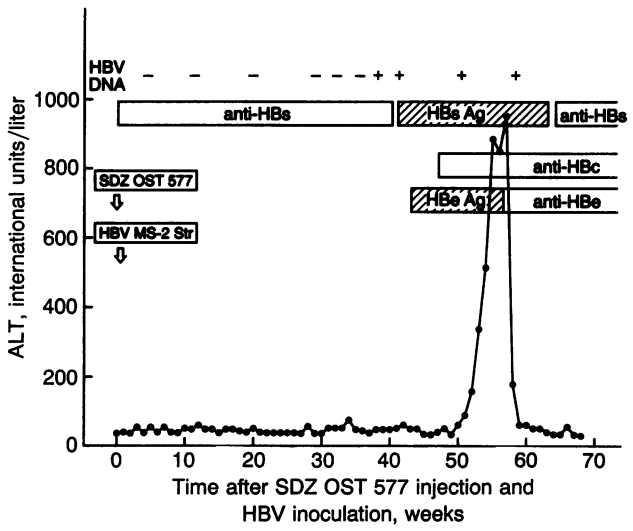

FIG. 2. Course of HBV infection in chimpanzee 1443. Serum and liver tissue HBV DNA was tested by a nested PCR assay. HBV antigens and antibodies in serum were scored as positive when the signal-to-noise (S/N) ratio was ≥2.1 by radioimmunoassay. ALT, alanine aminotransferase.

Thus, there was no evidence for the emergence of so-called "neutralization escape" mutants of HBV, even though the sequence of this virus would have permitted a nonlethal mutation of codon 145 of the surface gene without generating a stop codon in the polymerase gene.

Examination of Liver for Evidence of Viral Replication Before the Appearance of HBsAg in the Serum. Liver biopsies obtained from the chimpanzee during weeks 0, 1, and 37 were negative for HBcAg by immunofluorescence. The biopsy obtained on week 40 was borderline positive and biopsies obtained on weeks 42, 44, and 46 were positive for HBcAg. Liver biopsy specimens obtained at weeks 1, 4, 20, 32, and 37 after the virus challenge were tested by PCR for the presence of HBV DNA. All samples examined were negative for HBV DNA despite a positive signal in a control sample containing $10^{0.5}$ chimpanzee infectious dose equivalent of the challenge virus. The DNA of the control sample was extracted through the same procedures as liver tissue DNA. Therefore, we concluded that virus replication was not detectable in the liver until just prior to the appearance of HBsAg in the serum.

DISCUSSION

Chronic HBV infection is one of the commonest indications worldwide for liver transplantation because of its association with cirrhosis, end-stage liver disease, and hepatocellular carcinoma. However, the prognosis after orthotopic liver transplantation in such patients has been poor when compared to patients with other diseases, largely because of the recurrence of chronic hepatitis caused by HBV that persisted in the recipient and infected the grafted liver (27, 28). Attempts to prevent such recurrences, including the use of passive immunoprophylaxis, active immunoprophylaxis, and therapy with interferon α, either alone or combined, have not been successful in diminishing the recurrence of HBV-associated liver disease after transplantation. Although none of these therapies has demonstrated a convincing effect on the rate of recurrent HBV-associated disease, passive immunoprophylaxis with repeated high doses of HBIG (29) has been thought to be the method most likely to delay (30) or even to prevent (31, 32) recurrence of the disease. Recently, McMahon *et al.* (10) reported the results of treatment with the human anti-HBs monoclonal antibody studied herein. Among six patients with chronic HBV-related liver disease who underwent liver transplantation, three of the patients treated with multiple doses of SDZ OST 577 continuously during the peri- and post-transplantation periods did not have a reappearance of serum HBsAg or clinical hepatitis, and two of these patients have survived HBV-free for >1 year. In the remaining three patients, significant hepatitis did not recur but HBV strains that harbored genetic mutations in the *a* determinant did emerge. Presumably, the emergence of these *S* gene mutations of HBV was facilitated by the presence of anti-HBs monoclonal antibodies. Although the fine specificity of the binding of this monoclonal antibody to HBsAg has not been fully analyzed, its high affinity for HBsAg of both *adw* and *ayw* subtypes (9) and much reduced affinity for HBsAg containing amino acid mutations in the *a* determinant of the HBV strains that emerged during treatment (10) strongly suggest that the monoclonal antibody is directed against a region of the *a* determinant of HBsAg.

The study reported here was designed to assess the protective efficacy of human anti-HBs monoclonal antibody SDZ OST 577 *in vivo* under controlled conditions. Our results clearly demonstrated that preexposure prophylaxis with a single high dose of SDZ OST 577 failed to protect the chimpanzee from HBV infection and development of hepatitis but did markedly prolong the incubation period. This was confirmed by the finding that the nucleotide sequence of the

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ayw cons.     484 ATT CCA GGA TCA TCA ACC ACC AGC ACG GGA CCA TGC AGA ACC TGC ACG ACT 534
MS-2 inoc.        --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --C
MS-2 repl.        --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --C

ayw cons.     110 Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Thr Thr 126
MS-2 inoc.        -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -
MS-2 repl.        -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

ayw cons.     535 CCT GCT CAA GGA ACC TCT ATG TAT CCC TCC TGT TGC TGT ACA AAA CCT TCG 585
MS-2 inoc.        AT- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- ---
MS-2 repl.        AT- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- ---

ayw cons.     127 Pro Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser 143
MS-2 inoc.        Ile -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -
MS-2 repl.        Ile -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

ayw cons.     586 GAC GGA AAT TGC ACC TGT ATT CCC ATC CCA TCA TCC TGG GCT TTC GGA AAA 636
MS-2 inoc.        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MS-2 repl.        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

ayw cons.     144 Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys 160
MS-2 inoc.        -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -
MS-2 repl.        -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -
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FIG. 3. Nucleotide and predicted amino acid sequence of codon positions 110–160 of the *S* gene of HBV DNA recovered from the MS-2 challenge pool (MS-2 inoc.) and from serial serum samples from chimpanzee 1443 (MS-2 repl.). The sequences are aligned with the consensus sequence of HBV subtype *ayw* DNA (*ayw* cons.) (20–26). Dashes indicate identical residues.

a determinant in the HBsAg of the administered HBV and of the recovered virus from the chimpanzee's sera had identical sequences, indicating that the virus recovered from the chimpanzee was, indeed, the challenge virus and not a mutant virus that emerged as the result of environmental pressure.

The MS-2 strain of HBV was recovered by Krugman *et al.* (11) from natural infections of HBV at the Willowbrook State School, Staten Island, New York, and was subsequently used to infect volunteers (12). This strain has been evaluated extensively in chimpanzees and has served as a challenge virus for numerous studies of active and passive immunoprophylaxis. Active immunoprophylaxis with either plasma-derived or recombinant HBV vaccines against this strain (33–37), as well as synthetic peptides representing the *a* determinant of HBsAg (38), protected against infection and hepatitis caused by this strain of HBV. Passive immunoprophylaxis with HBIG against the MS-2 strain of HBV has been less successful: in most cases infection and hepatitis were not prevented (39, 40). Instead, the incubation period of HBV infection was prolonged. These results were similar to those obtained in a number of trials of HBIG for the prevention of hepatitis B in adults in the 1970s and 1980s (41–44), although immunoprophylaxis trials of HBIG in newborns were generally successful (45), especially when coupled with active immunoprophylaxis (46–48). Thus, the failure of HBIG and/or monoclonal anti-HBs antibodies to protect *in vivo* against the MS-2 strain of HBV is probably not due to the nucleotide change that replaces the amino acid Proline with isoleucine at position 127 in the MS-2 strain of HBV.

The majority of mutant HBV strains that have been recovered from failures of active or passive immunoprophylaxis have been found to have glycine replaced by arginine at the *S* gene codon position 145 (10, 49–51). However, other amino acid changes in the *a* determinant of HBsAg have been reported (10, 50–52). How many, if any, of these *S* gene mutants actually represent neutralization escape mutants is difficult to determine at present.

The present study was designed to examine the neutralizing capacity of an anti-HBs monoclonal antibody directed against the *a* determinant of HBsAg and to see if it facilitated the emergence of an *S* gene mutant virus. The dose of administered anti-HBs was quite high and exceeded 10 sample ratio units of RIA until the 33rd week of the study. A challenge dose of virus comparable to that used in previous studies of active immunoprophylaxis was administered 72 h after administration of the monoclonal antibody, at a time when maximum titers of the antibody should have been uniformly distributed throughout the body of the chimpanzee. Despite a prolongation of the incubation period by ≈500%, the monoclonal antibody did not completely neutralize the virus. Since the anti-HBs was present continuously at potentially protective levels during almost the entire incubation period, it seems clear that a single dose of SDZ OST 577 alone is incapable of efficiently neutralizing this wild-type strain of HBV.

A murine anti-HBs monoclonal antibody, thought also to be directed against the *a* determinant of HBsAg, did apparently neutralize an identical dose of the MS-2 strain of HBV and an *adr* strain of HBV, when the viruses were mixed with the monoclonal antibody and incubated *in vitro* before intravenous inoculation into chimpanzees (53). However, this monoclonal antibody was not tested for *in vivo* efficacy as described herein.

The ability to test for neutralizing antibodies against HBV remains a difficult procedure because the virus does not replicate in standard cell cultures, making *in vitro* neutralization studies in cell culture virtually impossible. *In vitro* neutralization of HBV, followed by inoculation of the antigen–antibody mixture into a chimpanzee, is feasible but very expensive and time consuming and may not accurately reflect the *in vivo* neutralizing capabilities of an antibody. Thus, the *in vivo* neutralization procedure employed in this study may be the only method at present for predicting the efficacy of monoclonal or polyclonal anti-HBs preparations in preventing HBV infection and hepatitis. This will be an impediment to studies of other human anti-HBs monoclonal antibodies that are emerging as the result of combinatorial library technologies for generating large numbers of monoclonal antibodies *in vitro*.

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