Vaccine Against Human Hepatitis B

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- A highly purified and inactivated vaccine was made of hepatitis B virus surface antigen. The vaccine was tested exhaustively for safety by ordinary procedures and additionally in chimpanzees and marmosets. It was highly potent and induced antibody in guinea pigs, grivet monkeys, and chimpanzees after three doses of vaccine were given subcutaneously. Chimpanzees given three doses of vaccine were protected against challenge with 1,000 chimpanzee-infectious doses of live human hepatitis B virus given intravenously in controlled studies. Tests of the vaccine for control of hepatitis B in man are to be carried out.

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THE PRESENT report describes the development and evaluation in chimpanzees of a subunit vaccine that protects them against hepatitis B or "serum hepatitis." Certain of the safety and potency test findings were presented earlier in a preliminary report.1

The hepatitis B (HB) virus has failed to date to multiply significantly in cell culture. What is known of the morphology of the virus has come mainly from studies by electron microscopy of the blood and liver of human beings infected with the virus.

Currently, the infectious HB virus is believed to be the so-called Dane particle2 that has been found in the serum of patients infected with the agent. The Dane particle is 42 mμ in diameter and consists of an inner core (nucleocapsid, HB,Ag) and an outer shell of glycoproteins on the virus surface (designated "hepatitis B surface antigen" or HB,Ag). Measuring 22 mμ in diameter and found to circulate in great abundance in the blood of certain persons who are infected with HB virus, HB,Ag2-5 is believed to be surface antigen that is produced in excess by the virus and that finds its way into the blood. There are two main serotypes of HB virus in the United States and they are designated type ad and type ay. Both are based on antigens present in the HB,Ag. The α antigen is common to both serotypes and is believed to be the primary immunologic determinant.

Krugman et al6 prepared a vaccine in which boiled, diluted plasma from a human carrier of HB,Ag stimulated antibody in human subjects and protected them against the disease. We have prepared a vaccine by using highly purified HB,Ag that was treated with formaldehyde solution, tested for safety and potency, and evaluated for protective efficacy in chimpanzees.

MATERIALS AND METHODS

In Vitro Assays.—Tests for HB,Ag were carried out by the Ausrria I or Ausrria II tests, by the passive hemagglutination test, or by the complement-fixation (CF) test.7 Tests for antibody against hepatitis B surface antigen (anti-HB) were performed by the Ausab or the passive hemagglutination assay. Complement-fixation tests for anti-HB8 were also performed. Hepatitis A antibody was assayed by the immune adherence (IA) test9 and assays for serum glutamic oxaloacetic transaminase and for serum glutamic pyruvic transaminase were performed by the Sigma-Frankel method.

Hepatitis B Vaccine Preparation, Lot 559.—Plasma pools were collected from four overtly healthy human donors who had hepatitis B antigenemia. Approximately 89% of the plasma was from donors with type ad HB virus and 11% from donors with type ay. A highly purified HB,Ag for vaccine preparation was obtained by isopyknic banding, rate-zonal separation,10 and chemical procedures. The purified antigen was adjusted to a concentration of 20μg/ml of protein and treated for 72 hours at 36 C with formaldehyde solution in a concentration ratio of 1:4,000. The treated material was the vaccine.

The vaccine was prepared and tested by procedures consistent with the existing standards of the Bureau of Biologies of the Food and Drug Administration.11 Tests for absence of live hepatitis A virus were carried out in white-moustached marmosets (Saginus mystax) as previously described.12 Conventional tests were employed to rule out presence of extraneous
human blood proteins and blood group substances.

Chimpanzees have been demonstrated to be susceptible to human hepatitis B following inoculation with the virus.\(^{14}\) Four chimpanzees, weighing 9.1 to 18.2 kg (20 to 40 lb) each, were selected for the safety tests of the vaccine based on absence in repeat tests of HB,Ag and antibody, on absence of elevation in transaminases, on absence of hepatitis liver histopathology (needle-biopsy specimens), and on the finding of a negative tuberculin reaction. Each chimpanzee was given 1.0 ml of lot 559 vaccine intravenously and, at the same time, six similarly selected control chimpanzees were given approximately 1,000 chimpanzee-infectious doses of human HB virus. The animals were bled just prior to injection, on the first, fourth, and seventh days after infection, and at weekly intervals, for a total of 40 weeks. Liver biopsy specimens were taken at monthly intervals for histopathologic examination for hepatitis. All six of the control chimpanzees had hepatitis as indicated by the findings for HB,Ag, transaminase elevation, antibody against the core antigen of the virus, and histopathologic findings. By contrast, none of the animals given the vaccine had positive reactions in any of the tests for hepatitis B, indicating lack of presence of infectious virus in the vaccine.

**RESULTS**

**Assays of Lot 559 Hepatitis B Vaccine for Immunizing Potency.**—Initially seronegative guinea pigs, grivet monkeys, and chimpanzees were each given three doses of the vaccine subcutaneously at 20µg per dose, as shown in Table 1. The chimpanzees were selected by the same criteria as described in the section on materials and methods. All of the guinea pigs, five of the six grivet monkeys, and five of the six chimpanzees had anti-

HB, in the amount shown in Table 1. This indicated adequate potency of the vaccine to permit protective efficacy tests in chimpanzees. Importantly, from the standpoint of safety, none of the six chimpanzees given the vaccine subcutaneously had any of the criteria for HB virus infection during the 16 weeks after vaccination, as described in the section on materials and methods, and this served as an additional test for safety of the vaccine. Furthermore, antibody against hepatitis A virus did not develop in any of the chimpanzees, indicating absence of live hepatitis A virus in the vaccine.

**Assay for Protective Efficacy of the Vaccine.**—The six chimpanzees that were given three doses of lot 559 vaccine and five unvaccinated control animals were all challenged with approximately 1,000 chimpanzee-infectious doses of live HB virus given intravenously. The animals were observed for 24 weeks for development of HB virus infection and the findings are shown in Table 2. The five control animals, all initially devoid of antibody against HB,Ag, had HB infection as indicated by tests for HB,Ag, transaminase elevation, and anti-HB, levels. Antibody against the surface antigen developed in three animals.

As noted in Table 1 and seen in Table 2, five of the six chimpanzees given the vaccine had anti-HB, by the time of challenge. None of the animals, including animal 6, which had no detectable antibody, had hepatitis B following challenge, as shown by the lack of antigenemia, transaminase elevation, and anti-HB. The one animal that did not develop antibody against surface antigen on vaccination did have such antibody following challenge.

**COMMENT**

The conventional safety and sterility tests served to rule out microbial contamination and contamination with possible exogenous viral agents present in the vaccine. The tests in marmosets\(^{15}\) ruled out possible presence of human hepatitis A virus. The tests with chimpanzees served to rule out the presence of live HB virus. Additional tests of lot 559 and two other lots of vaccine given in 100-ml volume intravenously to chimpanzees have not shown the presence of live virus. The relative sensitivity of man as compared with chimpanzees for reacting to HB virus is not known.

There are, however, other determinants of safety. The density gradient centrifugation used in the purification process separates the small, non-infectious 22-mµ HB,Ag particles used to prepare the vaccine from the 42-mµ infectious Dane particles. Further, the conditions in purification, exclusive of treatment with formaldehyde solution, were shown in our laboratories to be highly destructive of infectivity of all of a number of viruses tested. Thus, the procedures were shown to totally inacti-
vate highly infectious samples of representative examples of a wide number of virus families. These included vesicular stomatitis virus (rhabdovirus), Newcastle disease virus (myxovirus), reovirus (reovirus), Mongo virus (picornavirus), herpes simplex 1 (herpesvirus), infectious bronchitis virus (coronavirus), and human hepatitis A virus (likely an entero-virus-like virus). The highly purified and formaldehyde-treated HB virus proved very active in inducing antibody against HBsAg in guinea pigs, monkeys, and chimpanzees. Most important, the vaccine, given in three doses, was highly protective in preventing hepatitis in chimpanzees challenged by intravenous injection with live HB virus in infected human plasma. All of the six vaccinated chimpanzees, in five of which detectable levels of antihB, had developed following vaccination, were protected against development of antigenemia following challenge with the live virus, and none showed complement-fixation against core antigen. Antigenemia, aside from enzyme elevation, is considered to be the primary indication for infection with HB virus in these animals.

The demonstration of protection in chimpanzees against HB virus by administration of purified formaldehyde-treated HBsAg is consistent with the protection afforded human subjects given crude bovine human plasma in early experiments carried out by Krugman et al. The results of the tests in the present study give promise for eventual control of hepatitis B by a highly purified viral antigen vaccine.

The findings in the present study will be presented in greater detail. Animal studies of purified HB vaccine along similar lines are being carried out by Purcell et al (personal communication).


References


INTELLECTUAL DEVELOPMENT OF CHILDREN.—The evidence that children learn during the first years of life is irrefutable. That this early learning is the basis for later intellectual development is predicted by the principles of critical periods and neurological plasticity, and has been documented in human beings by educators and psychologists. This early learning primarily depends on the interaction of the child with his caretakers and his environment. During the first year of life, it is important that the child’s experiences are arranged to correlate with the emergence of new sensorimotor abilities and new interests in his environment. During the second and third years of life, the child’s language interests, curiosity, and emerging independence must be actively fostered by his caretakers. Child health care personnel are in a unique position to affect this crucial period of intellectual development through education of parents in role as teachers, and by the establishment of mother-child developmental clinics for high-risk children. Such an approach on a national level will likely prove a less expensive and more effective means of preventing educational incapacitation than the preschool programs now in effect.—Marquis P: Cognitive stimulation. Am J Dis Child 130:410-415, 1976.