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Overview of clinical studies with hepatitis B vaccine made by recombinant DNA

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Summary

The Merck, Sharp and Dohme hepatitis B vaccine formulated from HBsAg produced by a recombinant strain of *Saccharomyces cerevisiae* has proven to be highly immunogenic and safe. A 10 μg dose of the vaccine produced an anti-HBs response of ⩾ 10 IU/l in 91% or more of healthy adults who completed the three-dose regimen. Children responded well to all levels of vaccine antigen utilised but developed maximum anti-HBs titres with 5 μg doses. The age of the vaccine recipient affected responsiveness. Younger adults (20–29 years) responded more rapidly and with higher anti-HBs titres than did older adults (⩾ 50 years). Children responded faster and with higher anti-HBs levels than younger adults. Clinical reactions reported after vaccination were mild and transient.

Introduction

Human hepatitis B infection is a worldwide public health problem. As there is no effective treatment for hepatitis B infection, prevention is essential. A safe and effective hepatitis B vaccine composed of hepatitis B surface antigen (HBsAg) purified from the plasma of human carriers of the virus is commercially available. An attractive alternative to human plasma as a source of HBsAg is the use of recombinant DNA technology to effect synthesis of surface antigen by a culture of micro-organisms. HBsAg purified from fermentation cultures of a recombinant strain of the yeast *Saccharomyces cerevisiae* containing the gene for HBsAg has been formulated into a vaccine through adsorption on alum adjuvant.[1] Human clinical trials utilising vaccine made from HBsAg produced in yeast and purified by hydrophobic interaction chromatography followed by gel exclusion chromatography[2] were initiated in 1983. The results of several studies demonstrating the safety and immunogenicity of the recombinant vaccine in humans have been reported.[2–9] This paper presents a summary to date of the clinical and antibody responses obtained in studies with 1144 healthy adults and 79 children.

Methods

Informed written consent was obtained for all participants. Subjects entering studies described in this paper were in good general health and had not previously received any hepatitis B vaccine. They were initially seronegative for HBsAg, anti-HBc and anti-HBs and had a normal level of serum alanine aminotransferase. Pregnant women were excluded from the studies.

Vaccine was administered as a series of three injections. The first two

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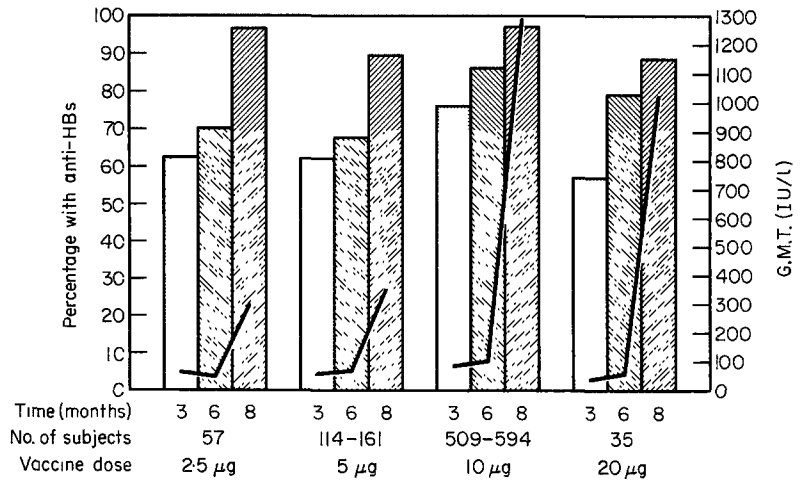

Fig. 1. Anti-HBs seroconversion rates and geometric mean titre (G.M.T.) among healthy adults vaccinated with varying doses of vaccine. Bars indicate seroconversion rates, lines are G.M.T.

injections were given a month apart followed by a third or booster injection at 6 months. All vaccinations were administered intramuscularly in the deltoid or, in the case of young children, the anterior lateral aspect of the thigh. Vaccine recipients or their parents were advised to record body temperature daily and to report any injection site discomfort or systemic complaints occurring within a 5-day period following each injection of vaccine.

Blood samples were taken at 0, 1, 3, 6 and 7–9 months following administration of the first dose of vaccine. Sera were tested for HBsAg, anti-HBc and anti-HBs using radioimmunoassay kits produced by Abbott Laboratories. Anti-HBs titres were determined in International Units/l (IU/l) as described by Hollinger *et al.*[10] A responder was defined as an initially seronegative individual who developed an anti-HBs titre $\geqslant$ 10 IU/l following vaccination.

Samples were also tested for antibody to yeast antigens using a radioimmune assay described previously.[2] Briefly, an extract of the parent strain of *S. cerevisiae* lacking the plasmid containing the gene for HBsAg was prepared by disruption of yeast cells in a homogeniser. The homogenate was clarified by centrifugation and filtration. The extract then was adsorbed to polystyrene beads. To perform the assay, diluted sera were incubated with the coated beads, the beads were washed and incubated with ^{125}iodine protein A. The protein A binds and labels any anti-yeast antibody of the IgG class that is on the bead. After several washes the radioactivity of the beads was counted and titres of antibody to yeast were determined from a standard curve derived from dilutions of a hyperimmune guinea-pig serum.

Results

Healthy individuals were vaccinated with 2·5, 5, 10 or 20 μg quantities of antigen in order to assess the degree of seroconversion and the height of antibody response. Figure 1 illustrates the serological responses in healthy adults

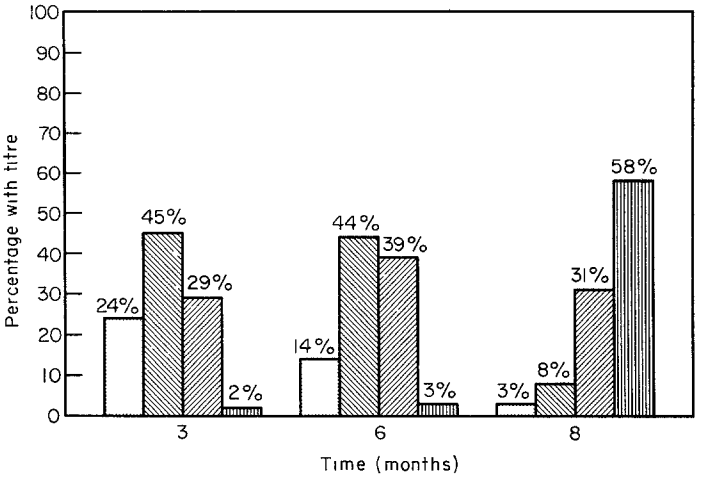

Fig. 2. Distribution of anti-HBs titres in international units/l (IU/l) among healthy adults receiving 10 μg doses of vaccine. □, < 10 IU/l; ▧, 10–99 IU/l; ▨, 100–999 IU/l; ▩, ⩾ 1000 IU/l.

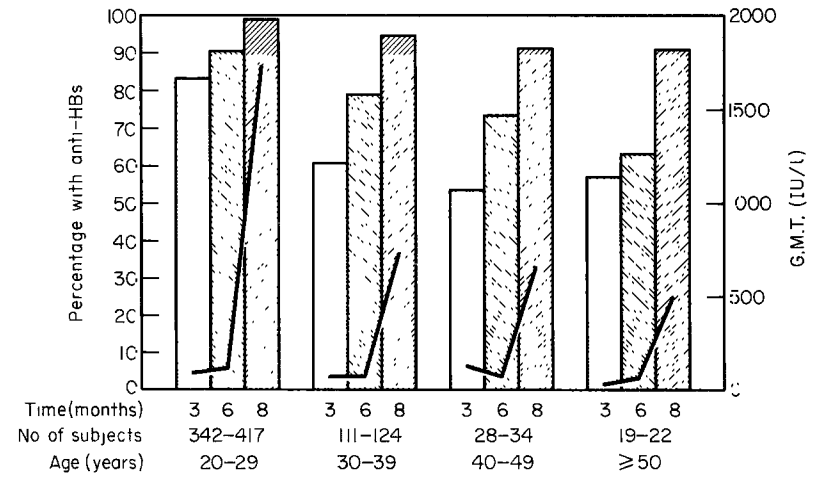

Fig. 3. Anti-HBs seroconversion rates and geometric mean titre (G.M.T.) by age among healthy adults receiving 10 μg doses of vaccine. Bars indicate seroconversion rates, lines are G.M.T.

for each of the doses given. The percentage of healthy adults seroconverting for anti-HBs (titre ⩾ 10 IU/l) at 3, 6 and 8 months was similar for all dosages studied. At 3 months (2 months after the second injection of vaccine) 57–76% of vaccine recipients developed antibody. By 8 months (2 months after the third injection of vaccine) 89–97% of vaccine recipients seroconverted. The geometric mean titre (G.M.T.) of vaccine responders at 8 months varied substantially with dosage. Higher G.M.Ts were obtained with 10 and 20 μg doses of vaccine (1286 and 1022 IU/l, respectively) than with the 2·5 and 5·0 μg doses (295 and 349 IU/l, respectively).

Because 10 and 20 μg doses of antigen gave similar serological responses,

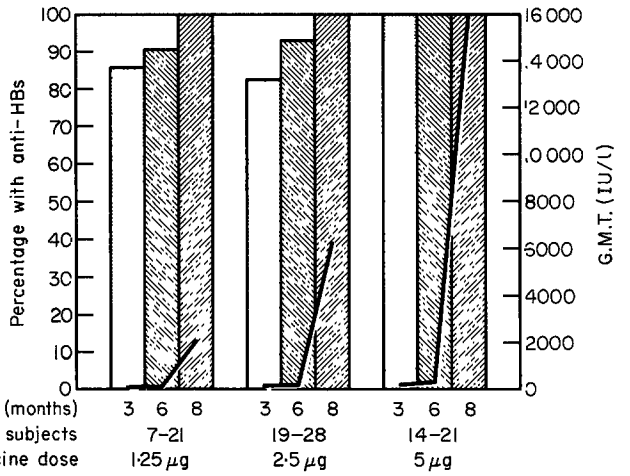

Fig. 4. Anti-HBs seroconversion rates and geometric mean titre (G.M.T.) among children vaccinated with varying doses of vaccine. Bars indicate seroconversion rates, lines are G.M.T.

most of the clinical trials in adults were with the 10 μg dose of vaccine. Figure 2 illustrates the distribution of anti-HBs titres *vs.* time among healthy adults who received 10 μg doses of vaccine. At 3 months, of 547 individuals evaluated, 76% had titres ⩾ 10 IU/l while 31% had titres ⩾ 100 IU/l and 2% had titres ⩾ 1000 IU/l. At 8 months, of 509 vaccinees evaluated, 97% had titres ⩾ 10 IU/l, while 89% had titres ⩾ 100 IU/l and 58% had titres ⩾ 1000 IU/l.

Healthy adults receiving 10 μg doses of vaccine ranged in age from 20 to 70 years. Figure 3 illustrates the antibody response of adults by age. Younger adults (20–29 years) showed a more vigorous antibody response to vaccine than did older subjects (⩾ 50 years). The effect of age was apparent both in seroconversion rates following only one or two injections of vaccine and in the G.M.Ts of responders following three injections of vaccine. Three 10 μg doses of the vaccine successfully seroconverted 91% of adults 50 years of age or older.

Early in the clinical trials in the U.S.A., children (1–12 years of age) were vaccinated with 1·25, 2·5 and 5 μg doses of recombinant vaccine in order to establish an appropriate immunogenic dose. Figure 4 summarises these results. Following vaccination, children developed antibody more rapidly than adults and did so even when given lower doses of the vaccine antigen. Eighty-two to one hundred per cent of children receiving 1·25–5 μg of vaccine antigen had an anti-HBs titre ⩾ 10 IU/l after two injections of vaccine. Two months after the third injection of vaccine 100% of the children had anti-HBs titres of ⩾ 10 IU/l at all doses of vaccine antigen. As with adults, G.M.Ts following three injections of vaccine varied directly with the dose of vaccine antigen. The G.M.T. at 8 months in recipients of 5 μg was 15966 IU/l; with 2·5 μg, 6230 IU/l; with 1·25, 2059 IU/l. Although seroconversion rates were excellent at all doses of vaccine antigen, the highest antibody titres were obtained with the 5 μg dose of vaccine.

The distribution of the anti-HBs titres *vs.* time among healthy children who received 5 μg doses is shown in Fig. 5. At 3 months 100% of the children had

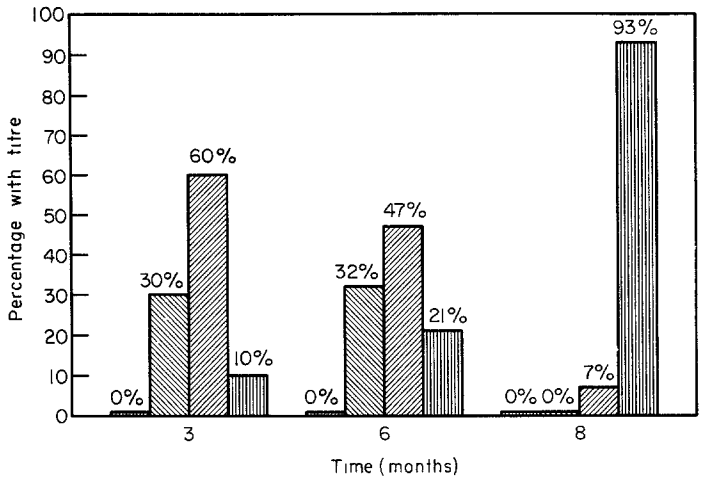

Fig. 5. Distribution of anti-HBs titres in international units/l (IU/l) among children receiving 5 μg doses of vaccine. □, < 10 IU/l; ▩, 10–99 IU/l; ▨, 100–999 IU/l; ▥, ⩾ 1000 IU/l.

titres ⩾ 10 IU/l. Of these, 30% had titres of 10–99 IU/l, 60% had titres of 100–999 IU/l and 10% had titres ⩾ 1000 IU/l. At 8 months 100% of the children had titres ⩾ 100 IU/l; of these, 93% had titres ⩾ 1000 IU/l.

Safety

The recombinant hepatitis B vaccine prepared in yeast has been well tolerated and has proven to be quite safe. When the clinical studies were first initiated, there had been a theoretical concern that administration of a vaccine prepared in yeast might lead to induction of allergic reactions to yeast proteins. In the trials to date there have been no reports of local or systemic reactions that were attributed to sensitisation to yeast proteins. To further evaluate this issue, sera from 133 vaccine recipients who had participated in several of the earlier clinical trials were assayed for anti-yeast IgG by the radioimmune assay procedure described in the methods section. Pre-vaccination and post-vaccination serum samples taken at 1, 3, 6 and 7–9 months were tested. One hundred per cent of individuals tested had anti-yeast IgG in both pre- and post-vaccination samples. The titres in pre-vaccination sera ranged from 12000 to 104000 antibody units. The anti-yeast IgG titre following vaccination fluctuated over time. When clinical reactions were compared to antibody titres, clinical reactions were not more frequent in persons with rises in yeast antibody titres.

Participants in the clinical trials recorded their temperature and any complaints they had for 5 days following each injection of vaccine. No serious adverse effects attributable to the vaccine have been reported in over 3000 individuals studied to date. All clinical complaints reported have been mild and transient.

Table I illustrates the percentage of healthy adults receiving 10 μg doses of vaccine who recorded any complaint during the 5-day period following each

Table I *Percentage of adults with a clinical complaint during a 5-day period following vaccination with 10 μg of vaccine*

Type of complaint	First injection (%) (n = 1144)	Second injection (%) (n = 1056)	Third injection (%) (n = 559)
Injection site*	18	13	20
Systemic†	17	11	11

* Soreness, pain, tenderness.
† Headache, fatigue/weakness, nausea, malaise.

Table II *Percentage of children with a clinical complaint during a 5-day period following vaccination*

Type of complaint	First injection (%) (n = 79)	Second injection (%) (n = 75)	Third injection (%) (n = 75)
Injection site*	3	3	1
Systemic†	18	15	8

* Soreness.
† Fatigue/weakness, diarrhoea, irritability.

vaccine injection. Complaints of soreness, pain or tenderness at the injection site were recorded by 18, 13 and 20% of adults following the first, second and third injections, respectively. Systemic complaints consisting most frequently of headache, fatigue, weakness or malaise were recorded by 17, 11 and 11% of adults following each injection, respectively. Temperature $\geqslant$ 38·3 °C was reported by 1% of adult vaccinees.

Mild transient local or systemic complaints were reported by less than 20% of all children vaccinated with either 1·25, 2·5 or 5 μg doses. As seen in Table II, after the first injection, 3% of the children had soreness at the injection site and 18% recorded systemic complaints such as fatigue, weakness, diarrhoea or irritability. The incidence of these complaints decreased for the second and third injection (15% and 8% respectively). After the first injection, 8% children developed a temperature of $\geqslant$ 38·3 °C while only 3% reported fever after the second and third injection of vaccine.

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