Hepatitis B Vaccination in Diabetic Patients

Randomized trial comparing recombinant vaccines containing and not containing pre-S2 antigen

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OBJECTIVE — To investigate the immunogenicity of two recombinant hepatitis B vaccines containing S antigen alone (Engerix B) or both S and pre-S2 antigens (GenHevac B) in diabetic patients.

RESEARCH DESIGN AND METHODS — Of the adult diabetic patients, 71 (26 IDDM, 45 NIDDM) were randomized to receive Engerix B or GenHevac B at 0, 1, 2, and 12 months in a single-blind clinical trial; if the antibody to hepatitis B surface antigen (anti-HBs) titers were <10 IU/l at month 4, a fourth injection of vaccine was given. A positive response was defined by anti-HBs titer ≥10 IU/l at month 13.

RESULTS — The anti-HBs response rate and the titers of anti-HBs did not differ significantly between the two types of vaccine. Overall, >90% of the patients responded at month 13. In patients vaccinated with GenHevac B, anti–pre-S2 antibodies appeared earlier than anti-HBs. The anti-HBs response tended to decrease with age ($P = 0.07$) and tended to be higher in IDDM patients than in NIDDM patients ($P = 0.06$). Metabolic control, as assessed by HbA_{1c} level, did not influence the response rate. The presence of the HLA DQ2 allele was associated with a low response.

CONCLUSIONS — A large majority of diabetic patients can be efficiently vaccinated against the hepatitis B virus using a booster dose at month 4. The choice of the vaccine (with or without pre-S2 antigen) appears to have little influence, if any, on the response rate.

Diabetic patients are known to have impaired defense mechanisms and to be at risk for nosocomial diseases (1). Some reports have stressed that hepatitis B virus markers were more frequent in diabetic subjects than in the healthy population (2,3), and cases of acute or chronic hepatitis B have still been described recently (4,5). It has been suggested that diabetic patients have a reduced immune response to hepatitis B vaccination compared with healthy subjects (6). This low response rate could be linked to the presence of DR3 and DR7 HLA alleles in IDDM patients as well as in the general population (6,7).

Hepatitis B vaccines were initially produced from plasma derived from hepatitis B surface antigen (HBsAg) chronic carriers. More recently, recombinant vaccines containing both S and pre-S2 antigens of the hepatitis B virus envelope were produced in mammalian cells. The addition of the pre-S2 antigen in vaccines has been shown to induce pre-S2 antibodies and could also enhance the response of the antibody to hepatitis B surface antigen (anti-HBs) in mice (8). Whether the addition of the pre-S2 component in vaccines reduce the number of low responders in humans remains controversial (9,10). The aim of this randomized trial was to compare the immunogenicity of hepatitis B recombinant vaccines containing and not containing pre-S2 antigen in diabetic patients and to study the genetic and clinical factors influencing the response.

RESEARCH DESIGN AND METHODS

Vaccines

The two vaccines used were the commercially available yeast-derived recombinant hepatitis B vaccine Engerix B from SmithKline and Beecham and the mammalian cell-derived recombinant hepatitis B vaccine GenHevac B from Pasteur-Merieux, both containing 20 μg of HBsAg. In addition, the second vaccine contained 20% of pre-S2 Ag.

Patients

The inclusion criteria were as follows: *1*) >18 years of age; *2*) diabetes defined by a fasting plasma glucose ≥7.8 mmol/l on two occasions and known for at least 1 year; *3*) normal hepatic and renal functions; *4*) no previous hepatitis B vaccination; and *5*) negativity for HBsAg, total antibody to hepatitis B core antigen (anti-HBc), anti-HBs, antibody to hepatitis C virus (anti-HCV), and antibody to human immunodeficiency virus (anti-HIV).

Of the diabetic patients, 82 entered this single-blind trial after giving informed written consent. The protocol had been approved by the Henri Mondor Hospital Ethic Committee. Patients were randomized to receive either the Engerix B (group E) or GenHevac B vaccine (group G). The vaccines were given in four single doses, administered in the deltoid muscle at 0, 1, 2, and 12 months. In addition, when the anti-HBs

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anti-HBs, antibody to hepatitis B surface antigen; ELISA, enzyme-linked immunosorbent assay; HBsAg, hepatitis B surface antigen.

Table 1—*Clinical characteristics of the diabetic patients studied*

	Engerix B	GenHevac B	P
n	36	35	
Male sex	12 (33.3%)	17 (48.6%)	NS
Age (years)	52.4 ± 14.3	46.0 ± 13.0	NS
Type of diabetes			
NIDDM	22	23	NS
IDDM	14	12	NS
Duration of diabetes (years)	15.0 ± 9.0	8.6 ± 4.7	<0.002
HbA_{1c} (%)	8.0 ± 2.0	7.7 ± 1.9	NS

Data are means ± SD for quantitative parameters.

titer was <10 IU/l at month 4, a fourth injection was performed within 10 days.

Of the patients, 11 of 82 (5 in group E and 6 in group G) were excluded without evidence of a link to the vaccination, except for 2 patients who complained of pain at the site of injection. Therefore, data from 71 patients were analyzed, 36 patients in group E and 35 patients in group G. Characteristics of the patients are presented in Table 1. Classification of diabetes type into IDDM and NIDDM was based on clinical criteria (11). There were no statistically significant differences in sex, age, type of diabetes, and metabolic control assessed by HbA_{1c} levels between the two treatment groups (Table 1). However, the duration of diabetes was significantly longer in patients vaccinated with Engerix B.

Evaluation of the anti-HBs response and detection of anti–pre-S2 antibodies

A positive response to vaccines was defined as the appearance of anti-HBs at a titer ≥10 IU/l. Anti-HBs were measured at 1, 2, 4, 6, 12, and 13 months (enzyme-linked immunosorbent assay [ELISA], Abbott Laboratories). The endpoint was a priori defined to be the response rate at month 13.

Anti–pre-S2 antibodies were detected by an ELISA technique using the pre-S2 synthetic peptide (amino acids 120–153) of ay subtype and peroxidase-labeled monoclonal antibody anti-human IgG (12). Results were considered positive when the absorbance value at 492 nm was more than two times that in negative control subjects.

HLA typing

HLA class I phenotypes were determined using the standard National Institutes of Health lymphocyte cytotoxicity test (13). HLA class II phenotypes were determined using genomic studies, according to the procedure described by Bidwell and Bignon (14), able to type the DR1–DR18 and DQ1–DQ9 specificities.

Statistical analysis

Sample size was calculated so that a difference of 25% in the anti-HBs response could be detected, with a reference response rate of 65%, using a two-tailed test ($\alpha = 0.05$, $\beta = 0.30$). With an anticipated dropout rate of 20%, a sample size of 80 patients was required.

Comparisons of categorical variables between treatments used χ^2 test and, for small samples, the binomial distribution. Antibody titers were not normally distributed. Therefore, results were expressed as medians and ranges, and differences between treatments were tested by Wilcoxon's rank-sum test.

Because of the relatively small size of the patient sample, the relationship between the presence of an HLA specificity and the anti-HBs response was assessed by using the broad HLA antigens (namely DR1–DR10 and DQ1–DQ4). Because the presence of DR3 or DR7 is known to be associated with a lower immune response (5,6), a *P* value at 0.05 was considered statistically significant for both these antigens. Concerning the comparisons with no a priori hypothesis (i.e., those performed for the 12 other broad HLA antigens), using the Bonferroni's method, a *P* value = 0.05/12 = 0.0042 was required for statistical significance.

RESULTS

Anti-HBs response to vaccines

The anti-HBs response rate and the titers of anti-HBs did not differ significantly between the two types of vaccines at any time of the study. According to our protocol, 17 patients in group E (47.2%) and 13 in group G (37.1%) received a booster dose at month 4 (NS, $P = 0.39$). With this booster dose, the anti-HBs response rate reached 91.5% overall, 94.4% in group E, and 88.6% in group G (NS, $P = 0.66$).

No difference in the distribution of the titers of anti-HBs was observed between vaccines at any time, with month 13: 2,320 (0–1,920,000) IU/l [median (range)] in group E, and 2,361 (0–94,448) IU/l in group G (NS, $P = 0.60$).

Anti–pre-S2 response to GenHevac B

In patients vaccinated with GenHevac B, anti–pre-S2 antibodies appeared earlier than anti-HBs antibodies (Fig. 1). At month 1, 22.9% of the patients had anti–pre-S2 antibodies and only 5.7% had anti-HBs antibodies ($P = 0.031$). At month 4, the difference disappeared, and at month 13, 88.6% of the patients were anti-HBs positive and only 68.6% were anti–pre-S2 positive ($P = 0.039$).

Influence of sex, age, duration, metabolic control, type of diabetes, and HLA phenotype on anti-HBs response

Since no statistically significant differences were observed between the responses to the two vaccines, the influence of patients' characteristics was studied in the whole population of patients.

The anti-HBs response was not statistically different in women and men (92.9 and 89.7%, respectively). The anti-HBs response tended to decrease with age. At month 13, the percentage of responders in patients younger than 40 years was 100%, whereas it was only 76.9% in patients older than 60 years ($P = 0.07$). Duration of diabetes did not significantly influence the response to vaccines. There was no significant correlation between HbA_{1c} level at baseline and anti-HBs titers at month 13 ($r = -0.15$, $P = 0.20$).

The anti-HBs response at month 4 tended to be higher in IDDM patients (65.4%) than in NIDDM (53.3%) patients ($P = 0.06$), but similar rates of response were observed at month 13: 92.3% in IDDM patients vs. 91.1% in NIDDM patients ($P = 0.95$), while a trend was still observed for higher titers of anti-HBs at month 13 in IDDM patients compared with NIDDM patients ($P = 0.06$).

Anti-HBs titers were compared at month 13, depending on the presence or absence of DR and DQ alleles. In HLA-DR7 patients, anti-HBs titers tended to be lower ($P = 0.07$), while they were not in HLA-

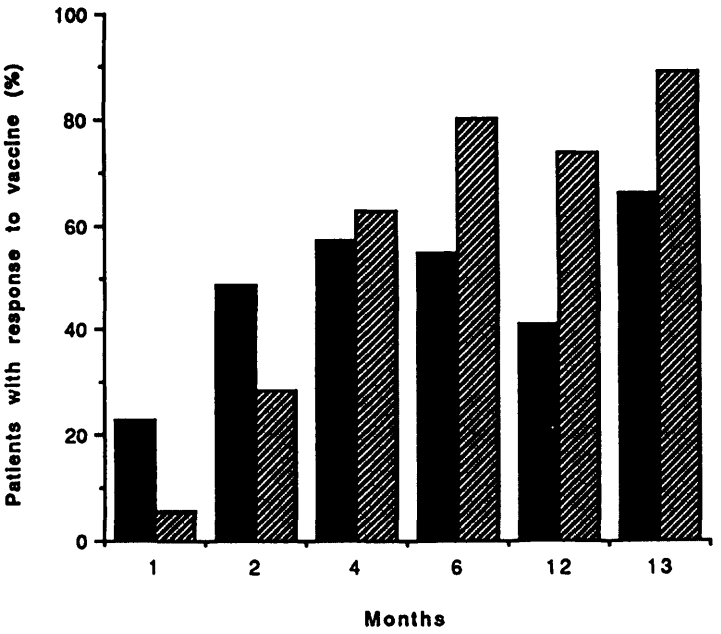

Figure 1—*Anti–pre S2 (■) and anti-HBs (▨) responses in patients vaccinated with GenHevac B.*

DR3 patients (P = 0.21). The allele most closely associated with a low response was DQ2 (P = 0.005), close to the level of statistical significance (see "Statistical analysis"). Five of the six subjects with no antibody response had the allele DQ2. No association was found between anti-HBs titers and the presence of the other HLA class II antigens or the HLA class I antigens.

CONCLUSIONS — Diabetic patients should be vaccinated against the hepatitis B virus (1,2,4,5). The present study indicates that diabetic patients are slow rather than low responders to the hepatitis B virus vaccine. Two months after the third injection, a response was observed in only 57.7% of the patients, whereas the response rate is ~95% in healthy subjects of similar age (15). To improve the response rates, a fourth dose of vaccine was given when the anti-HBs titer was <10 IU/l at month 4. The resulting rate of seroconversion was 81.7% at month 6 and 91.5% at month 13. Our protocol therefore provided an efficient protection in an a priori low responder population and suggests that a booster injection at month 4 is useful when no response has been obtained after the first three injections.

No difference in the anti-HBs production has been shown between the two hepatitis B recombinant vaccines containing and not containing the pre-S2 antigen, confirming previous data (10). However, after the GenHevac B vaccine, anti–pre-S2 antibodies appeared earlier than anti-HBs antibodies. An early appearance of anti–pre-S2 antibodies could lead to an efficient protection before the production of anti-HBs antibodies, as has been shown in chimpanzees (16).

In our study, the rate of anti-HBs response and the titers of anti-HBs antibodies tended to be higher in IDDM patients than in NIDDM patients. This may be due to the younger age of IDDM patients. There was so little overlap for age between the two types of diabetes that no adjustment could be performed to make any distinction between the respective effect of these two factors on the anti-HBs response. The HLA specificity most closely associated with a low anti-HBs response was the HLA-DQ2 antigen. It can be hypothesized from this finding that the previously described associations between a low response to hepatitis B vaccination and the presence of HLA-DR7 and DR3 alleles (6,7) are in fact due to a tight association with the DQ2 allele, which is in strong linkage disequilibrium with both DR3 and DR7.

In conclusion, this clinical trial indicates the following: *1*) diabetic patients are slow responders to hepatitis B vaccine and should be given a booster shot if no response has been obtained after the third injection; *2*) a 90% response rate can be achieved in diabetic patients; *3*) the choice between vaccines containing and not containing pre-S2 antigen appears to have a low influence, if any, on the response rate; and *4*) the presence of HLA-DQ2 allele is associated with a relatively low response rate.

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